THE

EXCRETION OF CREATININE BY THE

HUMAN KIDNEY.

by

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PART I. DETERMINATION OF CREATININE

Introduction

As will be discussed in greater detail in a later section of this thesis, creatinine has been widely recommended as a test substance in measuring, in man, glomerular filtration rate; and under other conditions, tubular excretory activity. The validity of such observations is, however, dependent on the accuracy of the chemical analysis. It is well known, however, that the usual chemical methods are unsatisfactory, since, for example, the conventional method (alkaline picrate) has been claimed to give high results for plasma, but not for urine. If this is the case, then the values calculated for UV/P are too low, and therefore the ratio of 1.0 accepted for C_{Cr}/C_{In} is incorrect. Further, if C_{In} really does measure glomerular filtration rate, it follows that C_{Cr} cannot also do so. To decide this controversial point, the investigation reported in this thesis was undertaken, and the first essential was to examine existing analytical methods, with the object of finding a specific method for the accurate determination of creatinine in plasma and urine.

DETERMINATION OF CREATININE

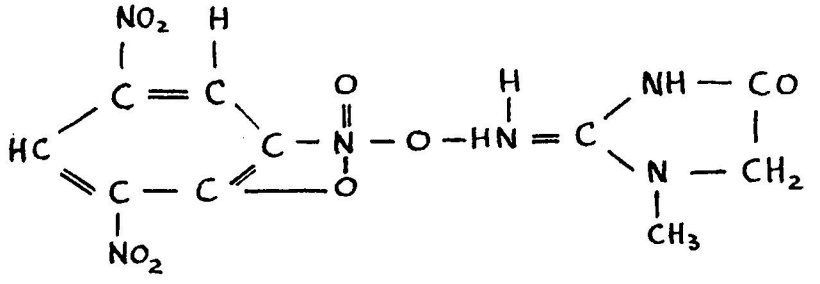
INTRODUCTION

The Jaffé Test.

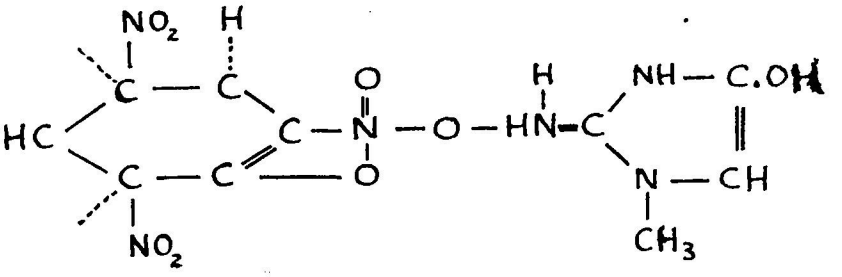
If a creatinine solution, to which has been added some aqueous picric acid, is rendered alkaline with sodium or potassium hydroxide, it develops, even in the cold, a colour varying with the concentration of creatinine from reddish-orange to deep blood-red (Jaffé 1886). The colour increases in depth on standing, and reaches a maximum in ten minutes. The rate of its development follows the law of a monomolecular reaction with a velocity constant (per minute) of 0.46 (Hunter and Campbell, 1917).

The red substance produced in the test was originally supposed to be picramic acid, i.e., amino-dinitrophenol, derived from the picric acid by reduction (van Hoogenhuyze and Verploegh, 1908). Benedict and Osterberg (1921), however, found that although the product of the reaction (isolated in a state of imperfect purity) approximated to picramic acid in elementary composition, it differed completely from the latter in physical properties and stability. When dry it formed a bright carmine powder, which if exposed to light, rapidly turned lemon-yellow, as though it were reoxidised to picric acid. By adding hydrochloric acid to a concentrated mixture of creatinine (1 mol.), sodium picrate (2.5 mols.), and a slight excess of sodium hydroxide,

Greenwald and Gross (1924, I) have obtained this substance in a state of purity, and have shown that when heated it is transformed into ordinary creatinine picrate. It would appear therefore to be merely a tautomeric form of the latter and the production of this tautomer would seem to be the essence of the Jaffé reaction. In support of this view Greenwald and Gross demonstrated that during the course of the reaction neither creatinine nor picric acid is destroyed, both being quantitatively recoverable at the moment when the colour has attained its maximum. Greenwald (1925, I) has shown that the only derivatives of creatinine which give the Jaffé reaction are those which (1) can form salts, and which (2) retain, with an unsubstituted - CH_2.CO-group, the possibility of a keto-enol tautomerism. He further concludes that the formation of the red tautomer involves changes in possibly all three nitro-groups of the picric acid and in at least one H in the <u>meta</u> position. Greenwald suggests that the relation between the ordinary yellow creatinine picrate and its red modification might be represented by the following formulae:-

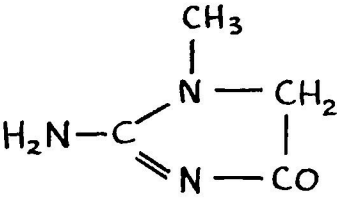


I. Yellow Creatinine Picrate.



II. Red Tautomer

In the second of these the dotted lines indicate that the exact position of the hydrogen and the disposition of the remaining valencies of the carbon atoms are not known. Plimmer (1925) on the other hand suggests the following structure for creatinine (III),



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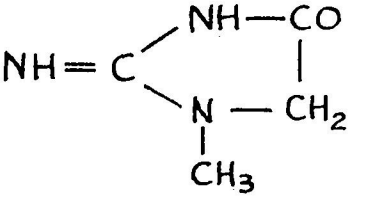


IV.

which he supposes to be changed by mineral acid into the one generally adopted (IV). Whatever the explanation may be concerning the mode of action of the picric acid, it is difficult to understand the reasoning behind the formulae of Greenwald and to accept either these or Plimmer's as being established. What does appear, however, to be demonstrated is that the reaction involves some kind of tautomerism, though whether this tautomeric change takes place

between two carbon atoms (keto-enol), or between nitrogen and carbon remains a matter of conjecture. Bolliger (1937) came to the conclusion that when different amounts of sodium hydroxide are added to an alcoholic solution of creatinine and picric acid four compounds are formed; the first contains (per mol. of creatinine) 1 mol. of picric acid and 2 mols. of sodium hydroxide; the second, third and fourth contain respectively 2½, 3 and 3½ mols. of sodium hydroxide for each molecule of creatinine. Compound 4 represents the maximum degree of saturation with alkali and was considered by Bolliger to be that chiefly responsible for the Jaffé reaction. Bonsnes and Taussky (1945) showed that the colour obtained in the Jaffé reaction is independent of the concentration of picric acid, at least over a fairly wide range, and that it is dependent upon the concentration of the alkali employed.

Out of a list of twenty-five mono, di- and trinitro aromatic compounds examined by Bitto (1892), Greenwald and Gross (1924, I) and Greenwald (1925, 2), the only ones other than picric acid, found to give any colour at all with creatinine were trinitro-benzene, trinitro-toluene and trinitrobenzoic acid (Greenwald and Gross). Even with these the reaction amounted to no more than a slight intensification of the colour produced by sodium hydroxide alone. In a private communication to Behre and Benedict (1936), Greenwald admits that he initially overlooked the

colour given by 3:5 dinitrobenzoic acid, owing to an incorrectly labelled sample of the material. He concludes by saying that further trials with the pure acid confirm the observations of Behre and Benedict, who successfully used the acid for the determination of creatinine in urine.

According to Riesser (1923) a distinct, if feeble reaction is given by meta-dinitro-benzene, with which however, von Bitto reported a negative result. In 1936, Langley and Evans and Behre and Benedict, both working independently, suggested the use of 3:5 dinitrobenzoic acid. Behre and Benedict, however, showed that although dinitrobenzoic acid gave a typical "creatinine colour" with urine, it yields an entirely different colour with blood and plasma filtrates. Bolliger (1936) and Langley and Evans (1936) have, however, suggested methods with the dinitrobenzoic acid reagent for determination of creatinine in blood filtrates. These investigators did not report any unusual difficulties in obtaining a colour match between blood filtrates and pure creatinine standards.

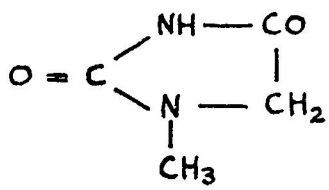
Behre and Benedict (1937) have used the reaction between creatinine, rubidium chloride and picric acid. This reaction yields an insoluble creatinine rubidium picrate and may be used to remove creatinine from pure solution. Applying this technique to blood filtrates, Behre and Benedict were unable to obtain typical precipitates, although such precipitates occurred upon addition of pure

creatinine to the filtrate. Gaebler (1937), however, found that a large portion of the chromogenic material in plasma ultra-filtrates could be precipitated by the rubidium-picric acid reagent, indicating that most of the chromogenic material in plasma is creatinine, and that the remainder constitutes a "non-creatinine chromogenic fraction."

The Enzymatic Method.

Miller and Dubos (1936-7) developed "adaptive" enzymes, produced from soil bacteria, to decompose creatinine, in order to overcome the non-specificity of the Jaffé reaction. The specificity of the creatinine oxidases was determined by testing them against a number of Jaffé-reactive substances related to or associated with creatinine. The addition of a methyl or acetyl group to the creatinine molecule inhibits the action of the enzyme. There are, however, many substances closely related to creatinine which do not give any colour with alkaline picrate, but are decomposed by the enzyme. The action of the enzyme on these Jaffé-negative substances was tested by the determination of a characteristic end-product, e.g. urea. (Miller and Dubos, 1937). Their results indicate that methyl-guanidine, and arginine are decomposed at about the same rate as creatinine. It would appear, therefore, that the ring structure of creatinine

is but a small factor in determining the specificity of the enzymatic action. The presence, however, of a guanidine-like unit in the molecule appears to be an important determinant of the specificity (Miller and Dubos, 1937). Miller and Dubos (1937) also showed that the replacement of one NH group by the O = C linkage, as in the change from creatinine and creatine to methylhydantoin (V) and methylhydantoic acid (VI), also prevents any reaction with the enzyme. Also, if the guanidine unit is non-methylated as in guanidine (VII), there is a marked reduction in the amount of urea produced, as compared with methylguanidine (VIII), and a similar effect accompanies a shift of the methyl group in creatinine from the 3 to the 5 position (IX) (the nomenclature is that used by Greenwald, 1925).



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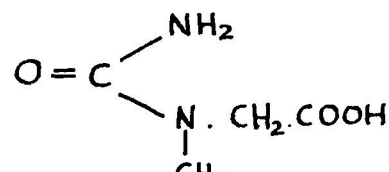

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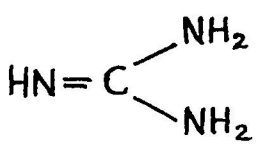

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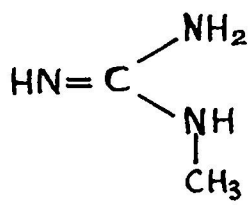

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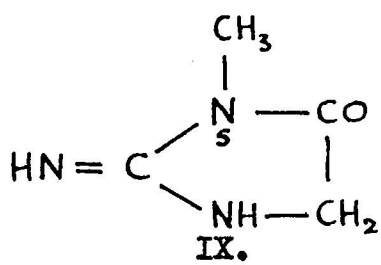


IX.

The 3:5 dinitrobenzoic acid reaction.

In 1936, Langley and Evans found that dinitrobenzoic acid gave a brilliant garnet-red colour with creatinine in alkaline solution, but that the acid alone gave no colour in alkaline solution. This acid, therefore, appeared to be unique in this respect, since many other nitro compounds, e.g. trinitrobenzoic acid, when dissolved in sodium carbonate solution give a pale yellow solution of the sodium salt, but give a deep red colour with excess sodium hydroxide. Any acid, which behaves in this way towards sodium hydroxide, is obviously unsuitable as a colorimetric reagent in place of picric acid.

The results of Langley and Evans, however, were very far below those obtained by the ordinary alkaline picrate method, even when one makes an allowance for the non-creatinine fraction. To confirm and substantiate their results, Langley and Evans analysed blood filtrates before and again after elution of the creatinine from Lloyd's reagent, by the use of magnesium oxide, as recommended by Gaebler (1930). They again found that before adsorption there was a big discrepancy in the results by the two methods, but that after adsorption and elution from Lloyd's reagent the results showed good agreement. This indicated that very nearly true creatinine values in blood could be obtained directly from the analysis of a blood filtrate; in contra-distinction to alkaline picrate which always

gives too high a value. On the other hand, Miller and Dubos (1937), using the Langley and Evans method, and the ordinary alkaline picrate method, showed that the two reagents gave the same results, when used with the more accurate spectrophotometric technique.

Since similar and even identical techniques had yielded entirely conflicting results in the hands of different workers, it was decided to re-examine the method of Langley and Evans, and to compare it with the alkaline picrate method of Miller and Dubos (1937). It was also deemed advisable to repeat the work of Miller and Dubos (1937), using the enzyme, since their experiments were carried out on American subjects. As a final check on the dependability and specificity of the methods, further simultaneous determinations in serum, by all three methods were considered necessary, in order to have a complete and comparative check of all the methods.

EXPERIMENTAL

Purification of Picric Acid

Hunter and Campbell (1916) noticed that a picric acid solution, if exposed to the action of light, came to contain in a steadily increasing quantity, a substance which gives a deep red colour upon treatment with alkali. A similar deterioration of picric acid in solution may be produced by heat (Wilson and Plass, 1917) and possibly by other agencies. The chromogenic substance thus producible has been attributed to an impurity or impurities in the picric acid (Folin and Doisy, 1917). Any picric acid solution containing such material will give a simulated Jaffé reaction, the intensity of which bears no relation to the quantity of creatinine that may be present. It is necessary, therefore, in connection with all colorimetric methods to purify the picric acid.

As a criterion applicable to any picric acid which it is proposed to use in creatinine determinations, Folin and Doisy (1917) suggest the following test. To 20.0 ml. of a 1.2 per cent solution of the acid add 1.0 ml. of 10 per cent NaOH and allow to stand for fifteen minutes, then compare the mixture in a Klett or Dubosq colorimeter with the original picric acid solution. If the latter is set at 20 mm., the former must not read less than 10 mm., and

must remain unaltered after twenty-four hours. The picric acid used throughout this investigation was purified according to the method suggested by Cole (1944). The alkaline picrate when tested gave a reading of 12 mm. on the colorimeter.

Cole's Method

The picric acid was recrystallized twice from boiling water, filtering the hot saturated solution through a hot Buchner funnel. This product was found to conform to the test of Folin and Doisy (1917).

Purification of 3:5-dinitrobenzoic acid.

Purification of the acid was carried out as recommended by Langley and Evans (1936). 50.0 g. were dissolved in 100 ml. of boiling 80 per cent alcohol. The solution was boiled for a few minutes and then cooled to about 5°C, whereupon crystals formed within a few minutes. After about half an hour at 5°C, the crystals were filtered off, and again dissolved in 100 ml. of boiling 80 per cent alcohol, cooled and filtered. For use, a third recrystallisation was done in order to remove any remaining yellow impurity. The purified acid was very pale yellow in colour and melted at 204.5°C (capillary tube).

Preparation of Reagents.

6 per cent Sodium Dinitrobenzoate.

30.0 g. of the acid were suspended in 420 ml. of doubly distilled water, and 80.0 ml. of 10 per cent sodium carbonate were added. When no more of the acid dissolved, the solution was filtered and was ready for use.

10 per cent Sodium-Dinitrobenzoate.

20.0 g. of dinitrobenzoic acid were suspended in 150.0 ml. of water, and 50.0 ml. of 10 per cent sodium carbonate added, filtered.

Picric Acid.

A saturated solution was made by adding an excess of the dry purified acid to 1,000 ml. of double distilled water. For use in the determination of creatinine the saturated acid was filtered through a Whatman No. 1 filter paper.

10 per cent Sodium Hydroxide (A.R.)

50.0 g. of sodium hydroxide pellets were dissolved in an appropriate quantity of double distilled water, allowed to cool, and made up to 500 ml. with double distilled water. This solution does not need to be free of carbonate.

10 per cent Sodium Tungstate (A.R.) ($Na_2WO_4 \cdot 2H_2O$)

100.0 g. of sodium tungstate were added to 1,000 ml. of double distilled water. The pH was adjusted so that phenol phthalein turned a permanent pale pink.

2/3 N. Sulphuric Acid (A.R.)

Method of Preparation of Suspension of Washed Cells (Culture NC)

The NC culture was maintained in a condition of great activity by weakly transfers into the following medium: 0.5 per cent creatinine, 0.5 per cent NaCl, M/40 pH 7.0 phosphate buffer in tap-water (Miller and Dubos, 1937).

Two litres of culture medium were made at a time, and distributed in 200 ml. quantities into large medical flats. This quantity gave a shallow layer of 3.0 cm. in depth when the medical flat was laid on its side. Two drops of the active culture were used to inoculate 200 ml. of the medium. The culture was then incubated for 48 hours at 37°C. (Allinson, 1945). According to Allinson this gives a more active enzyme preparation than the original method of Miller and Dubos, since only half the creatinine has been metabolised, and the soil bacteria are therefore in a more active state of multiplication. Miller and Dubos, on the other hand, incubated their culture for 72 hours, i.e., until all the creatinine had been metabolised. The cells are then separated by centrifugation, washed once with distilled water, and re-suspended in an amount of distilled water equal to 1/40 the volume of original culture medium, i.e. 50.0 ml. (Miller and Dubos, 1937).

Phosphate Buffer (Green) pH 7.0.

136.0 g. dihydrogen potassium phosphate are dissolved in the requisite quantity of 2M potassium hydroxide and diluted to one litre with distilled water.

Methods

The results of Langley and Evans (1936) using their own method with dinitrobenzoic acid as the colorimetric reagent, were very far below those obtained by the ordinary alkaline picrate method, even when one makes an allowance for the non-creatinine chromogenic material. On the other hand, Miller and Dubos (1937) using Langley and Evans' Method, including their method for the purification of the dinitrobenzoic acid, obtained results in close agreement with those obtained by the alkaline picrate method. They suggest that the discrepancy might be due to errors involved in the measurement of the colour, especially in the results of Langley and Evans who used a colorimeter; Miller and Dubos used a Pulfrich spectrophotometer. Whatever the reason, it was deemed advisable to repeat the work, using a Diffraction Grating Spectrophotometer (Unicam S.P. 350) at a wave length of 520 mμ. If the error was due to bad colorimetry, then the modern Unicam Spectrophotometer should eliminate it, since all readings are independent of the human factor.

Preliminary experiments indicated that 520 mμ was the most suitable wavelength at which to measure the picric acid and dinitrobenzoic acid colours, since they both strongly absorb above 510 mμ, and the colour due to the creatinine does not absorb much light above 540 mμ.

RESULTS

TABLE I.

Values for Creatinine Chromogenic Material on Serum Filtrates Obtained from Simultaneous Analysis by Alkaline Picrate and Dinotrobenzoic Acid Methods.
1 in 5 Folin-Wu tungstate filtrates

	Alkaline Picrate (Miller and Dubos)		Dinitrobenzoic Acid (Langley and Evans)	
No.	Scale Reading on S.P. 350	mg/100 ml. as creatinine	Scale Reading on S.P. 350	mg/100 ml. creatinine
1.	0.105	1.05	0.074	1.05
2.	0.105	1.05	0.072	1.04
3.	0.106	1.06	0.074	1.05
4.	0.103	1.03	0.074	1.05
5.	0.104	1.04	0.072	1.04
6.	0.104	1.04	0.074	1.05

These results indicate that dinitrobenzoic acid is no more specific than alkaline picrate. It was felt, however, that if the concentration of the two acids, or more important, the concentration of the alkali and the volume of filtrate, were altered, then it might be possible to obtain a method which would tend to depress temporarily the development of the non-creatinine chromogen.

The volume of serum filtrate and the reagents were selected arbitrarily, so that the determination could be carried out in the special test-tube (25 mm. diameter) for use with the spectrophotometer. Two methods were used as follows:-

Method I. To 5.0 ml. of serum filtrate is added 4.0 ml. of saturated picric acid solution and 1.0 ml. of 5 per cent sodium hydroxide.

METHOD I.

(Calibration Curve on S.P. 350)

The Standard Creatinine Solution contained 5.0 mg. per 1000 ml.

Amount of Creatinine (µg.) per 5.0 ml. of solution	0	5.0	10.0	15.0	20.0	25.0
ml. standard creatinine containing 5 µg/ml.	0	1.0	2.0	3.0	4.0	5.0 ml.
Water	5.0	4.0	3.0	2.0	1.0	0 ml.
Readings on S.P. 350	0	0.049	0.090	0.134	0.175	0.218
	0	0.045	0.090	0.134	0.180	0.222
Mean of two Readings	0	0.047	0.090	0.134	0.177	0.220

Method II. To 5.0 ml. of Serum filtrate is added 4.0 ml. of 6 per cent dinitrobenzoic acid and 1.0 ml. of 5 per cent NaOH.

METHOD II.

(Calibration Curve on S.P. 350)

The Standard Creatinine Solution contained 5.0 mg. per 1000 ml.

Amount of Creatinine (µg.) per 5.0 ml. of solution	0	5.0	10.0	15.0	20.0	25.0 ml.
ml. standard creatinine containing 5 µg/ml.	0	1.0	2.0	3.0	4.0	5.0 ml.
Water	5.0	4.0	3.0	2.0	1.0	0 ml.
Readings on S.P. 350	0	0.035	0.064	0.094	0.126	0.155
	0	0.033	0.063	0.094	0.125	0.153
Mean of two Readings	0	0.034	0.0635	0.094	0.1255	0.154

TABLE II

Values for Creatinine Equivalent of Chromogenic Material in Serum Filtrates Obtained from Simultaneous Analyses by Alkaline Picrate (Method I) and Dinitrobenzoic Acid (Method II) Methods.

1 in 5 Folin-Wu tungstate filtrates

Results calculated as creatinine

	Alkaline Picrate			Dinitrobenzoic Acid		
No.	Scale Reading on S.P.350	µg/5 ml. filtrate	mg/100 ml.	Scale Reading on S.P.350	µg/5 ml. filtrate	mg/100 ml.
1.	0.085	9.0	0.90	0.048	7.6	0.76
2.	0.085	9.0	0.90	0.054	8.6	0.86
3.	0.088	9.4	0.94	0.053	8.5	0.85
4.	0.092	9.8	0.98	0.052	8.4	0.84
5.	0.084	9.0	0.90	0.050	8.0	0.80
6.	0.093	9.8	0.98	0.053	8.5	0.85
7.	0.088	9.4	0.94	0.057	9.1	0.91
8.	0.082	8.7	0.87	0.050	8.0	0.80
9.	0.082	8.7	0.87	0.050	8.0	0.80
10.	0.090	9.6	0.96	0.055	8.8	0.88
Average			0.92	Average		0.83

The results in Table II indicated that the dinitrobenzoic acid reagent gave figures lower than those yielded by picric acid, the difference being of the same order of magnitude as the non-creatinine chromogenic material revealed by the enzyme method. Hence by altering the concentration of the alkali and the volume of serum filtrate, it might be possible to dispense with the use of the enzymatic method of Miller and Dubos (1937). A final answer to this problem could only be obtained however, by comparing these methods with the specific enzymatic method of Miller and Dubos, who have shown that creatinine derivatives, some very closely related to creatinine, are not acted upon by the enzyme. (Experimental proof is given in Table III to show that the enzyme does decompose the whole of the creatinine in a serum filtrate, and also creatinine added to serum).

Enzyme Method of Miller and Dubos.

Miller and Dubos' Method depends on the determination of the creatinine equivalent of the fluid with alkaline picrate before and after incubation with the enzyme preparation; the difference represents the true creatinine.

Preparation of Serum Filtrates.

The tungstic acid method of Folin and Wu (1919) was used. The only modification being that the serum dilution was 1:5 instead of the usual 1 in 10, so as to obtain a

colour which could be measured with greater accuracy, especially the interfering chromogen. To 4 volumes of serum were added 12 volumes of distilled water, 2 volumes of ten per cent sodium tungstate and 2 volumes of 2/3 N. sulphuric acid. The mixture was shaken thoroughly, allowed to stand for two or three minutes and filtered through a washed Whatman No. 1 filter paper.

Colour Development.

The Jaffé reaction was carried out at a constant temperature (20-21°C). One volume of alkaline picrate (5 volumes of picric acid plus 1 volume of 10 per cent sodium hydroxide) was added to two volumes of the solution to be analysed. The tubes (6" x 1") were shaken, placed in a water bath at 20-21°C, which was kept in the dark, and the optical density measured after 10 minutes in a spectrophotometer at a wave length of 520 mμ. All the estimations carried out in connection with the renal 'clearance' of creatinine were done on an S.P. 500 Spectrophotometer (Unicam) using 30.0 mm. cuvettes, at a wave length of 520 mμ and a slit-width of 0.03.

(a) Determination of Total Chromogenic Substance.

To 8.0 ml. of the serum filtrate are added 4.0 ml. of alkaline picrate and the mixture is allowed to react for 10 minutes, at 20°C, in the dark. The creatinine equivalent is then determined in the spectrophotometer at 520 mμ.

with 25.0 mm. test tubes. The blank contains 8.0 ml. of water and 4.0 ml. of alkaline picrate.

Calibration Curve

The Standard Creatinine Solution contained 3.0 mg. per 1000 ml.

Amount of Creatinine (µg) per 8.0 ml. of solution	0	6.0	12.0	18.0	24.0 µg.
ml. Standard Creatinine Solution containing 3.0 µg/ml.	0	2.0	4.0	6.0	8.0
Water	8.0	6.0	4.0	2.0	0 ml.
Readings on S.P. 350	0	0.035	0.068	0.106	0.139
	0	0.036	0.070	0.108	0.142
Mean of two Readings	0	0.0355	0.069	0.107	0.140

(b) Determination of Non-Creatinine Chromogenic Material.

2.0 ml. of the enzyme preparation is added to 10.0 ml. of the serum filtrate. After the addition of 0.5 ml. of molar phosphate buffer, pH 7.0, made according to Green (1933), the mixture is incubated at 37°C in an unstoppered 100 ml.

Erlenmeyer flask (Miller and Dubos, 1937) for 60 minutes. After incubation, the bacterial cells are removed by centrifugation at very high speed for half an hour. 4.0 ml. of alkaline picrate is added to 8.0 ml. of the clear supernatant fluid, and the mixture is allowed to react for 10 minutes in the dark, at room temperature. The creatinine is then determined in the spectrophotometer (Unicam S.P. 350). A control sample of 10.0 ml. of water, 2.0 ml. of enzyme preparation, and 0.5 ml. of buffer is treated in the same way and serves as the balancing solution in the spectrophotometer.

(c) Calculation of True Creatinine Concentration.

The concentration of the residual chromogenic substance (b) is corrected for the dilution. This value subtracted from the total chromogenic material obtained by (a) gives the true creatinine concentration.

According to Miller and Dubos (1937) the chief errors in their method come from the measurement of the residual chromogenic substance. If this value is large, the true creatinine is obtained by the subtraction of two relatively large numbers, and the error in such a case may be fairly large. They suggest that the determination of the residual chromogenic substance should be done in triplicate so as to overcome (1) any differences due to turbidity and (2) errors due to visual incompetence. In the present investigation this procedure was slightly modified, because it was found

by experience that the almost clear supernatant of the enzymic extract was as active as the mixed suspension of bacterial cells. This modified procedure in technique gave a clear solution after incubation and centrifuging, unhindered by any turbidity. It has, in fact, reduced such a possible error very considerably, and well below 5 per cent which is accredited to the Miller and Dubos method. Miller and Dubos used a Pulfrich spectrophotometer, which is dependent on human measurements, in the present investigation a diffraction grating spectrophotometer, and later, a quartz spectrophotometer were used. The error obtained with such refined instruments is therefore considerably smaller. The determination in urine is more precise because the residual chromogenic material is almost invariably a small fraction of the total.

To decide finally whether the true creatinine concentration could be determined with absolute certainty it was necessary to do some preliminary experiments on serum and plasma and also on serum to which had been added creatinine. If the true creatinine values agreed both before and again after the added creatinine, then it was almost certain that all the creatinine had been decomposed. The results of a typical experiment are given in Table III, but in this particular experiment, plasma containing glucose-citrate mixture was used. After this work had been completed, it was found that an autoclaved glucose-citrate mixture reacted

with alkaline picrate, and therefore accounted for the high results in this experiment.

Recoveries from Serum using Dinitrobenzoic Acid as the Colorimetric Reagent.

0.5 mg. per 100 ml. creatinine added to serum, mean of six determinations.

1 in 2.5 Folin-Wu filtrate.

	Before added creatinine		After added creatinine			
Serum No.	Scale Reading	µg/5 ml. filtrate	Scale Reading on S.P. 350	µg/5 ml. filtrate	µg/5 ml recovered	% Recovery
1.	0.110	17.7	0.170	27.6	9.9	99.0
2.	0.106	17.1	0.166	27.0	9.9	99.0
3.	0.110	17.7	0.170	27.6	9.9	99.0
1 in 5 Folin-Wu Filtrate.						
4.	0.050	9.0	0.077	14.0	5.0	100.0
	1.0 mg. per 100 ml. Creatinine added to Serum					
5.	0.047	8.0	0.103	18.0	10.0	100.0

Recoveries from Serum using Dinitrobenzoic Acid and Alkaline Picrate as the Colorimetric Reagents

0.5 mg. per cent Creatinine added to Serum.

Mean of Six Estimations.

1 in 2.5 Folin-Wu Filtrate.

Dinitrobenzoic Acid (Method II)

Serum No.	Scale Reading on S.P. 350	µg/5 ml. filtrate	Scale Reading on S.P. 350	µg/5 ml. filtrate	µg/5 ml. recovered	% Recovery
1.	0.095	15.2	0.155	25.1	9.9	99.0
2.	0.100	16.1	0.161	26.0	9.9	99.0

Alkaline Picrate (Method I)

1.	0.184	20.2	0.255	28.8	8.6	86.0
2.	0.190	21.0	0.268	29.6	8.6	86.0

Recoveries from Serum - Comparison of Three Methods.

1 in 5 Folin and Wu Filtrate — 0.75 mg. per cent creatinine added to serum.

Dinitrobenzoic Acid (Method II)

No.	Before: Scale Reading on S.P. 350	Before: µg/5 ml. filtrate	After: Scale Reading on S.P. 350	After: µg/5 ml. filtrate	µg/5 ml. recovered	% Recovery
3.	0.045	8.0	0.084	15.5	7.5	100.0

Alkaline Picrate (Method I)

No.	Before: Scale Reading on S.P. 350	Before: µg/5 ml. filtrate	After: Scale Reading on S.P. 350	After: µg/5 ml. filtrate	µg/5 ml. recovered	% Recovery
4.	0.075	8.3	0.127	14.1	5.8	77.4

Recoveries from plasma using the alkaline picrate method under controlled conditions.

1 in 5 Folin-Wu tungstate filtrate in all cases, subsequent dilutions being indicated by the figures in brackets.

Plasma No. 1 (Total Protein = 4.5 g./100 ml.)

	Reading	µg./ 8 ml.	mg./ 100 ml.	Average	% Recovery
Endogenous Level					
1.	.268	20.4	1.27		
2.	.267	20.3	1.27	1.27	-
3.	.267	20.3	1.27		
1.0 mg. per cent creatinine added (1 in 2 dilution)					
1.	.230	17.6	2.20		
2.	.235	18.0	2.24	2.25	98.0
3.	.237	18.1	2.26		
10 mg. per cent creatinine added (1 in 10 dilution)					
1.	.236	18.0	11.25		
2.	.236	18.0	11.25	11.25	100.0
3.	.236	18.0	11.25		
50 mg. per cent creatinine added (1 in 50 dilution)					
1.	.213	16.3	51.01		
2.	.215	16.4	51.25	51.17	99.8
3.	.215	16.4	51.25		

Plasma No. 2 diluted approximately 1 in 5 with citrate.

(Total protein = 5.27 g/100 ml.)

	Reading S.P.500	µg/ 8 ml.	mg./ 100 ml.	Average	% Recovery
Endogenous Level					
1.	.262	20.0	1.25		
2.	.270	20.5	1.28	1.27	-
3.	.270	20.5	1.28		
1.0 mg. per cent creatinine added (1 in 2 dilution).					
1.	.228	17.4	2.18		
2.	.233	17.8	2.22	2.21	97.5
3.	.233	17.8	2.22		
50 mg. per cent creatinine added (1 in 50 dilution).					
1.	.215	16.4	51.25		
2.	.215	16.4	51.25	51.25	100.0
3.	.215	16.4	51.25		

Serum No. 1 (Total Protein = 4.5 g./100 ml.)

	Reading S.P. 500	µg/ 8 ml.	mg/ 100 ml.	Average	% Recovery
Endogenous Level					
1.	.237	18.1	1.13		
2.	.237	18.1	1.13	1.13	-
3.	.238	18.2	1.13		
10 mg. per cent creatinine added (1 in 10 dilution).					
1.	.228	17.45	10.90		
2.	.230	17.6	11.0	10.97	99
3.	.230	17.6	11.0		
50 mg. per cent creatinine added (1 in 50 dilution).					
1.	.214	16.35	51.06		
2.	.214	16.35	51.06	51.06	99.86
3.	.214	16.35	51.06		

Serum No. 2 (Total Protein = 7.58 g./100 ml.)

Endogenous Level					
1.	.261	19.9	1.24		
2.	.261	19.9	1.24	1.24	-
3.	.261	19.9	1.24		
10 mg. per cent creatinine added (1 in 10 dilution).					
1.	.230	17.6	11.0		
2.	.230	17.6	11.0	11.08	98.75
3.	.235	18.0	11.25		
50 mg. per cent creatinine added (1 in 50 dilution).					
1.	.210	16.05	50.25		
2.	.212	16.20	50.62	50.49	98.5
3.	.212	16.20	50.62		

Recoveries from Urine using the Alkaline Picrate Method under controlled conditions.

	Dilution	Reading S.P.500	µg/ 8 ml.	mg./ 100 ml.	Average	%
Endogenous Level						
1.	1/200	.067	5.1	12.75		
2.	1/200	.068	5.15	12.87	12.83	-
3.	1/200	.068	5.15	12.87		
100 mg. Creatinine added per 100 ml. urine						
1.	1/1000	.120	9.1	113.5		
2.	1/1000	.120	9.2	115.0	114.5	101.5
3.	1/1000	.121	9.2	115.0		
500 mg. creatinine added per 100 ml. urine						
1.	1/5000	.106	8.1	506.25		
2.	1/5000	.106	8.1	506.25	506.25	98.72
3.	1/5000	.106	8.1	506.25		
1000 mg. creatinine added per 100 ml. urine						
1.	1/10000	.108	8.20	1025.0		
2.	1/10000	.107	8.15	1018.75		
3.	1/10000	.107	8.15	1018.75	1018.75	100.7
4.	1/10000	.107	8.15	1018.75		
5.	1/10000	.106	8.10	1012.50		

TABLE III

To show that under the conditions specified the enzyme does decompose all the creatinine in a serum filtrate, using the enzymatic method of Miller and Dubos and Alkaline Picrate as the colorimetric reagent.

1 in 5 tungstic acid filtrates.

	Before enzyme			After enzyme		
Serum	Scale Reading on S.P. 350	µg in 8 ml filtrate	mg per cent	Scale Reading on S.P. 350	µg. in 8 ml. filtrate	mg. per/ 100 ml.
1.	0.21	36.0	2.25	0.132	22.5	1.75
	0.21	36.0	2.25	0.132	22.5	1.75

0.5 mg. per 100 ml. creatinine added to serum

	Before enzyme			After enzyme		
Serum	Scale Reading on S.P. 350	µg in 8 ml. filtrate	mg. per cent	Scale Reading on S.P. 350	µg. in 8 ml.	mg. per/ 100 ml.
1.	0.255	44.0	2.75	0.132	22.5	1.75
	0.255	44.0	2.75	0.132	22.5	1.75

TABLE IV

To show that the enzyme does decompose all the creatinine in a serum filtrate, using the method of Langley&Evans with the enzyme and 3:5 dinitrobenzoic acid as the colorimetric reagent.

1 in 5 Folin-Wu tungstic acid filtrates.

	Before enzyme			After enzyme		
Serum	Scale Reading on S.P. 350	µg. in 8 ml. filtrate	mg. per cent	Scale Reading on S.P. 350	µg. in 8 ml. filtrate	mg. per/ 100 ml.
1.	0.072 0.072	16.4 16.4	1.04 1.04	0.010 0.010	2.2 2.2	0.17 0.17
2.	0.074 0.074	16.8 16.8	1.05 1.05	0.010 0.010	2.2 2.2	0.17 0.17

0.5 mg. per 100 ml. creatinine added to serum

	Before Enzyme			After Enzyme		
Serum	Scale Reading on S.P. 350	µg. in 8 ml. filtrate	mg. per cent	Scale Reading on S.P. 350	µg. in 8 ml. filtrate	mg. per/ 100 ml.
1.	0.107 0.107	24.8 24.8	1.55 1.55	0.010 0.010	2.2 2.2	0.17 0.17
2.	0.107 0.107	24.8 24.8	1.55 1.55	0.010 0.010	2.2 2.2	0.17 0.17

When 2 ml. of a standard creatinine solution containing 1 mg. per 100 ml. together with 6 ml. of water, plus 1 ml. of $^{2}/_{3}$ N acid and 1 ml. of sodium tungstate was incubated for one hour with the enzyme, no creatinine remained.

The results of four further experiments together with four controls also confirmed the belief that the enzyme is active in the presence of tungstic acid.

TABLE V.

True Creatinine in Urine from Normal Individuals

		Total Chromogenic Material		Residual Chromogenic Material after incubation with enzyme		True Creatinine	
No.	Dilution	Scale Reading	mg/ 100 ml.	Scale Reading	mg/ 100 ml.	mg/100 ml.	% of Apparent
1.	1 in 200	0.260	110	0.016	8.1	101.9	93
2.	1 in 200	0.300	120	0.012	6.25	113.75	95
3.	1 in 500	0.305	300	0.015	18.7	281.3	94
4.	1 in 500	0.352	350	0.015	18.7	331.3	95
5.	1 in 200	0.224	90	0.014	6.9	83.1	92
6.	1 in 500	0.166	165	0.004	4.7	160.3	97
7.	1 in 500	0.260	257	0.008	10.0	247.0	96
8.	1 in 500	0.218	218	0.010	12.5	205.5	95
9.	1 in 500	0.175	175	0	0	175.0	100
10.	1 in 500	0.214	212	0	0	212.0	100
Average							96

TABLE VI.

True Creatinine in Serum of Normal Individuals using the Method of Miller and Dubos and the Diffraction Grating Spectrophotometer S.P.350

	Total Chromogenic Material			Residual Chromogenic Material after incubation with enzyme			True Creatinine	
No.	Scale Reading	µg. in filtrate	mg/ 100 ml.	Scale Reading	µg. in filtrate	mg/ 100 ml.	mg/ 100 ml.	% of Apparent
1.	0.110	18.6	1.10	0.013	2.1	0.16	0.94	85
2.	0.105	17.8	1.05	0.017	2.8	0.22	0.83	79
3.	0.103	17.4	1.03	0.020	3.2	0.25	0.78	76
4.	0.109	18.4	1.09	0.014	2.3	0.18	0.91	83
5.	0.117	20.0	1.17	0.014	2.3	0.18	0.99	84
6.	0.107	18.2	1.07	0.018	3.0	0.23	0.84	78
7.	0.114	19.2	1.14	0.013	2.1	0.16	0.88	77
8.	0.112	19.0	1.12	0.013	2.1	0.16	0.96	86
9.	0.100	17.0	1.00	0.014	2.3	0.18	0.82	82
10.	0.124	21.0	1.24	0.020	3.2	0.25	0.99	80
11.	0.113	19.2	1.13	0.013	2.1	0.16	0.97	86
12.	0.110	18.6	1.10	0.020	3.2	0.25	0.85	77
13.	0.082	13.8	0.82	0.013	2.1	0.16	0.66	80
14.	0.120	20.0	1.20	0.015	2.4	0.19	1.01	84
15.	0.105	17.6	1.05	0.020	3.2	0.25	0.80	76
16.	0.123	20.6	1.23	0.015	2.4	0.19	1.04	84
17.	0.115	19.2	1.15	0.020	3.2	0.25	0.90	78
18.	0.114	19.0	1.14	0.017	2.8	0.22	0.92	78
Average			1.10			0.20	0.89	81

Values for Creatinine Equivalent of Chromogenic Material in Serum Filtrates Obtained from Simultaneous Analyses by Dinitrobenzoic Acid, Alkaline Picrate and Miller and Dubos Methods.

(mg. per 100 ml. Serum).

Serum	Dinitro-benzoic Acid Method II.	Alkaline Picrate Method I.	Alkaline Picrate (Miller and Dubos)		
			Total mg. per 100 ml.	Residual mg. per 100 ml.	True mg. per 100 ml.
1. Pooled	0.054 = 0.86	0.072 = 0.80	0.093 = 0.92	0.017 = 0.22	0.70
2. "	0.055 = 0.88	0.070 = 0.78	0.093 = 0.92	0.011 = 0.14	0.78
3. "	0.055 = 0.88	0.079 = 0.88	0.092 = 0.91	0.013 = 0.16	0.75
4. "	0.052 = 0.84	0.091 = 1.00	0.106 = 1.06	0.017 = 0.22	0.84
5. "	0.046 = 0.74	0.087 = 0.96	0.105 = 1.05	0.015 = 0.19	0.86
6. "	0.050 = 0.80	0.095 = 1.06	0.105 = 1.05	0.015 = 0.19	0.86
7. "	0.055 = 0.88	0.092 = 1.02	0.105 = 1.05	0.016 = 0.20	0.85
8. Not Pooled	0.050 = 0.80	0.085 = 0.94	0.098 = 0.98	0.014 = 0.17	0.81
9. "	0.050 = 0.80	0.072 = 0.80	0.092 = 0.92	0.013 = 0.16	0.76
10. "	0.045 = 0.72	0.070 = 0.78	0.080 = 0.80	0.014 = 0.17	0.63
11. "	0.056 = 0.90	0.094 = 1.04	0.105 = 1.05	0.013 = 0.16	0.89

TABLE VIII

Values for Creatinine Equivalent of Chromogenic Material in Serum Filtrates obtained from Simultaneous Analyses by Dinitrobenzoic Acid and Miller and Dubos Alkaline Picrate Methods, Applying the Enzyme to both Methods.

1:5 Folin-Wu Tungstate Filtrates (S.P. 500)

Serum	Dinitrobenzoic Acid			Alkaline Picrate		
	Total mg. Per 100 ml.	Residual mg. per 100 ml.	True mg. per 100 ml.	Total mg. per 100 ml.	Residual mg. per 100 ml.	True mg. per 100 ml.
Pooled	0.072 = 0.88	0.018 = 0.20	0.68	0.173 = 0.92	0.037 = 0.22	0.70
"	0.076 = 0.91	0.017 = 0.22	0.69	0.174 = 0.93	0.037 = 0.22	0.71
"	0.072 = 0.88	0.016 = 0.24	0.64	0.170 = 0.91	0.034 = 0.21	0.70,
"	0.072 = 0.88	0.017 = 0.25	0.63	0.171 = 0.91	0.033=0.19	0.72
Not Pooled	0.077 = 0.88	0.017 = 0.25	0.68	0.187 = 0.99	0.031 = 0.19	0.80
"	0.070 = 0.85	0.007 = 0.10	0.75	0.180 = 0.96	0.023 = 0.16	0.80

True Creatinine in Fresh Plasma and Erythrocytes, determined Simultaneously, of Normal Individuals Using the Method of Miller and Dubos

Plasma (1 in 5 Folin-Wu Tungstic Acid Filtrate)

	Total Chromogenic Material			Residual Chromogenic Material after incubation with enzyme			True Creatinine	
No.	Scale Reading on S.P. 350	µg. in filtrate	mg/ 100 ml.	Scale Reading on S.P. 500	µg. in filtrate	mg/ 100 ml.	mg/ 100 ml.	% true creatinine
1.	0.186	16.9	1.06	0.023	2.1	0.16	0.90	
2.	0.162	14.7	0.92	0.023	2.1	0.16	0.76	
3.	0.168	15.3	0.94	0.028	2.5	0.19	0.75	
4.	-	-	-	-	-	-	-	
5.	-	-	-	-	-	-	-	
pp6.	-	-	-	-	-	-	-	
Average			0.97			0.17	0.80	
				Red Blood Cells (1 in 10 Folin-Wu Filtrate)				
1.	0.128	11.6	1.45	0.037	3.3	0.52	0.93	64
2.	0.113	10.2	1.28	0.048	4.3	0.67	0.61	47
3.	0.107	9.7	1.21	0.045	4.0	0.62	0.59	48
4.	0.100	9.0	1.12	0.065	6.0	0.47	0.65	58
5.	0.127	11.5	1.44	0.090	8.1	0.63	0.81	56
6.	0.100	9.0	1.12	0.028	2.5	0.39	0.73	65
Average			1.27			0.55	0.72	57

Adsorption of Creatinine from Pure Solution and from Tungstic Acid Filtrates.

The method was the same as that used by Danielson (1936). To 10.0 ml. of the creatinine solution or to 10.0 ml. of a serum filtrate in a centrifuge tube were added 1.0 g. of acid-washed kaolin (B.D.H.) and 1.0 ml. of 2 N oxalic acid. The tube was stoppered and placed in a shaking machine for 15 minutes, centrifuged, and the supernatant liquid drained off into a 6" x 1" pyrex tube. 1.0 ml. of 2N soda was then added; the mixture was allowed to stand for a few minutes, and centrifuged again to get rid of the fine precipitate which formed on standing. 8.0 ml. of this supernatant fluid was then taken and 4.0 ml. of alkaline picrate added, and allowed to stand in the dark, at room temperature for 10 minutes.

Serum No.	Before Kaolin Treatment: Scale Reading	Before Kaolin Treatment: µg. per 8.0 ml. filtrate	Before Kaolin Treatment: mg./ 100 ml. apparent creatinine	After Kaolin Treatment: Scale Reading	After Kaolin Treatment: µg. per 8.0 ml. filtrate	After Kaolin Treatment: mg./ 100 ml.	After Kaolin Treatment: mg./ 100 ml. true creatinine	After Kaolin Treatment: % of apparent creatinine
1.	0.166	14.7	0.92	0.032	2.8	0.21	0.71	77
1.	0.166	14.7	0.92	0.032	2.8	0.21	0.71	77
Control containing 30.0 µg/100 ml. creatinine				0	0	0	0	-
2.	0.170	15.1	0.95	0.037	3.2	0.24	0.24	75.5
2.	0.170	15.1	0.95	0.045	3.9	0.29	0.66	70
2.	0.170	15.1	0.95	0.043	3.7	0.28	0.67	72
2.	0.170	15.1	0.95	0.035	3.0	0.22	0.73	78
Control containing 30.0 µg/100 ml. creatinine				0	0	0	-	-
Average						0.24	0.69	73

DISCUSSION.

After a thorough trial of the two colorimetric reagents, i.e. 3:5-dinitrobenzoic acid and alkaline picrate, using established and modified techniques, it became increasingly evident that if a specific colorimetric method was to be established it would have to be compared with the specific enzymatic method of Miller and Dubos (1937). The results, using this method, show that on an average 80 per cent of the total chromogenic material in serum represents true creatinine; Miller and Dubos (1937) found on an average 80-100 per cent. The results for urine, show that practically all the chromogenic material in urine is true creatinine; thus confirming the results of Miller and Dubos. The results also confirm the findings of Hunter and Campbell (1917) that erythrocytes contain considerable amounts of non-creatinine material. The total chromogenic material in erythrocytes considerably exceeds that in plasma, although the fraction represented by 'true creatinine' corresponds to that found in the plasma. The results would indicate that the true creatinine is distributed between cells and plasma in relation to their respective water contents.

Early experiments with dinitrobenzoic acid, using a modified Langley and Evan's technique indicated that it might be more specific than alkaline picrate, and the

results suggested that it might be possible to dispense with the use of the enzyme. However, when the two reagents were tested simultaneously with the enzymatic method of Miller and Dubos (1937), and using a quartz spectrophotometer, it became apparent that dinitrobenzoic acid is no more specific than picric acid.

The results of the three methods on serum filtrates finally prove that dinitrobenzoic acid is no more specific than alkaline picrate. Miller and Dubos (1937) did show, however, that dinitrobenzoic acid is more specific when tested on various compounds closely related to creatinine. Further, the investigation has proved that if creatinine is to be determined with any degree of accuracy then the enzymatic method of Miller and Dubos (1937) is the only available method for its estimation.

AN INVESTIGATION INTO THE IDENTIFICATION OF THE INTERFERING CHROMOGEN.

The lack of specificity of the alkaline picrate reaction has been criticized ever since it was first introduced by Jaffé in 1886. Although it is true that many compounds give the reaction in vitro, it is equally true to say that most of these compounds are not present in body tissues and fluids (Beard, 1941). It has long been supposed that a chromogenic substance is present in blood which is not creatinine. In 1928 Gaebler and Keltch stated that both normal blood and blood from nephritic subjects contain chromogenic substances other than creatinine; the same view was emphasized by Bohn and Hahn (1933). Hayman, Johnston, and Bender, studying the rate of colour development in a manner similar to that used by Hunter and Campbell (1917), found that material eluted from Lloyd's reagent which had been shaken with a trichloracetic acid filtrate of serum gave curves identical with those from pure creatinine solutions. A small amount of colour did develop in the serum filtrate after it had been treated with Lloyd's reagent, but the rate of colour development of this small fraction was, however, quite different from that given by creatinine. Since this was a constant finding,

Hayman et al. interpreted it as indicating the presence in normal blood of a small fraction of material, other than creatinine, which reacts with alkaline picrate. Danielson (1936) found that about 80 per cent of the chromogenic material in whole blood and plasma filtrates can be adsorbed by and eluted from kaolin, suggesting that 20 per cent of the so-called "apparent creatinine" is due to the interfering chromogen or chromogens.

Experimental

There are many normal constituents of blood which might be expected to interfere with the alkaline picrate reaction. Glucose and fructose reduce alkaline picrate solutions rapidly enough when heated, but at room temperature give no immediate reaction. Glucose solutions containing 100, 200, 300, 400 and 500 mg. per cent diluted 1 in 5 with distilled water give no appreciable colour with alkaline picrate at room temperature. Gerard and Tupikova (1938) showed that in resting oxygenated tissue the total creatinine was partitioned between phosphocreatine, free creatine, and residual chromogen in the following percentages: nerve 40, 40, 20; muscle 50, 44, 4; and brain 20, 60, 20. They believe that the residual chromogen is not creatine and that it may be bound to protein. A 1.0 mg. per cent creatine solution diluted 1 in 5 with water fails

to give any colour with alkaline picrate even after several hours. After standing overnight there is only a very slight intensity of colour, and then merely as a consequence of its gradual transformation by the alkali into creatinine. Experiments with serum ultra-filtrates, using the enzymatic method of Miller and Dubos (1937) indicate that the residual chromogen is not bound to protein, since some colour is always obtained with alkaline picrate after incubating with the enzyme under conditions which permit complete destruction of pure creatinine in similar amounts. The value for a serum - ultra-filtrate diluted 1 in 5 with water, is in fact identical with that for the tungstic acid filtrate of serum or plasma; i.e. circa 0.20 mg. per cent. A 1.0 mg. per cent solution of ascorbic acid diluted 1 in 5 with water fails to give any colour with alkaline picrate. This would appear to be rather surprising in view of its powerful reducing properties. A 5.0 mg. per cent solution of glutathione, diluted 1 in 5 with water, also fails to give a colour with alkaline picrate, also does a 10.0 mg. per cent solution of glutamic acid, diluted 1 in 5 with water.

Table 1 Showing How Various Normal Constituents of the Blood React with Alkaline Picrate.

Substance	Concentration mg. per 100 ml.	Reading on S.P. 500	mg. per 100 ml. as creatinine
Glucose	100	0	0
"	200	0	0
"	300	0	0
"	400	0	0
"	500	0	0
Creatine	0.5	0	0
"	0.5	0	0
Ascorbic Acid	1.0	0	0
"	1.0	0	0
Glutathione	5.0	0	0
"	5.0	0	0
Uric Acid	3.0	0	0
"	3.0	0	0
Plasma-Ultra-filtrate after incubation with the enzyme	-	0.025	0.18
	-	0.025	0.18
Acetone	5.0	0	0
"	5.0	0	0
Sodium Aceto-Acetate+	4.0	0.063	0.35
" "	4.0	0.063	0.35
" "	2.0	0.030	0.17
" "	2.0	0.030	0.17
Sodium β-hydroxy-butyrate	4.0	0	0
	4.0	0	0
Phenol	5.0	0	0
"	5.0	0	0
Catechol	5.0	0	0
"	5.0	0	0
Citric Acid	2.0	0	0
" "	2.0	0	0
Pyruvic Acid	1.0	0	0
" "	1.0	0	0

+ The aceto-acetic acid was prepared from the ester which was purified once by redistillation in vacuo.

Preparation of Aceto-Acetic Acid.

Method.

100 ml. of N soda (1/10 gram. mol.) and 300 ml. of distilled water were poured into a 500 ml. volumetric flask. 13.0 g. (1/10 gram. mol.) of aceto-acetic ester was weighed out and added to the contents of the flask. The weighing bottle was rinsed out three times with distilled water and the volume made up to 500 ml. with more distilled water. The flask was then stoppered, inverted several times and allowed to stand at room temperature for 24 hours. According to Cole this time is sufficient for the complete hydrolysis of the ester. This solution then corresponds to a 2.0 g. per 100 ml. solution of aceto-acetic acid. For use in the following experiments 1.0 ml. of the 2.0 g. per 100 ml. solution was diluted to a litre, i.e. a 2.0 mg. per cent solution of aceto-acetic acid or 1.13 mg. per 100 ml. solution calculated as acetone.

Experiment 1. 2.0 ml. of the 2.0 mg. per cent solution of aceto-acetic acid was added to 8.0 ml. of distilled water. 8.0 ml. was then taken and 4.0 ml. alkaline picrate added, allowed to stand at room temperature in the dark for 10 minutes. The resulting colour was then compared against a "reagent blank" (8.0 ml. water and 4.0 ml. alkaline picrate) in the S.P. 500 spectrophotometer.

Results

No.	Scale Reading on S.P. 500	µg. in 8.0 ml. of solution	mg. per 100 ml. calculated as creatinine
1.	0.030	2.7	0.17
2.	0.030	2.7	0.17
3.	0.027	2.5	0.14
4.	0.027	2.5	0.14
5.	0.027	2.5	0.14
6.	0.027	2.5	0.14

Experiment 2. From acid solution creatinine is adsorbed by charcoal (Sudendorf and Lahrmann, 1915; Folin and Denis, 1916; Behre and Benedict, 1922) and unlike creatine by kaolin (Greenwald and McGuire, 1918) and Lloyd's reagent (Folin and Berglund, 1922, I; Behre and Benedict, 1922). The work of Danielson (1936) besides substantiating the findings of these workers, also indicates that the interfering chromogen is not adsorbed by kaolin and Lloyd's reagent. If, therefore, aceto-acetic acid is the interfering chromogen, then it should not be adsorbed by either of the two adsorbing reagents. The method was similar to that used by Danielson (1936).

2.0 ml. of a 2.0 mg. per cent solution aceto-acetic acid was added to 8.0 ml. of water, simulating a 1 in 5 serum

filtrate. To 10.0 ml. of this solution in a centrifuge tube were added 1.0 g. of acid-washed kaolin and 1.0 ml. of 2 N oxalic acid. The tube was stoppered and placed in a shaking machine for 5 minutes, centrifuged, and the supernatant liquid poured off; and 1.0 ml. of 2 N NaOH added. To 8.0 ml. of the solution, 4.0 ml. of alkaline picrate was added. The blank was treated in the same way except that water was substituted for the aceto-acetic acid solution.

Results

No.		Scale Reading on S.P. 500	µg. aceto-acetic acid in 8.0 ml. of solution.	mg. per 100 ml calculated as creatinine
1.	Treated with kaolin	0.029	2.4	0.15
2.	"	0.027	2.4	0.15
3.	"	0.027	2.4	0.15
4.	Untreated	0.027	2.4	0.15
5.	"	0.027	2.4	0.15

Conclusion. The results indicate that aceto-acetic acid is not adsorbed by kaolin.

Experiment 3. For use with the enzyme 10.0 ml. of a 1 in 5 dilution of the 2.0 mg. per cent solution aceto-acetic acid was incubated with 0.5 ml. phosphate buffer solution and 2.0 ml. of the enzyme preparation, for one hour at 37°C. The solution was cooled, and centrifuged at a very high speed for 15 minutes, 8.0 ml. of the supernatant liquid was taken for the colorimetric estimation with 4.0 ml. alkaline picrate and allowed to stand for 10 minutes in the dark, at room temperature.

Results.

No.	Scale Reading on S.P. 500	µg. in 8.0 ml. of solution as creatinine	mg. per 100 ml. calculated as creatinine
1.	0.020	2.0	0.16
2.	0.020	2.0	0.16
3.	0.020	2.0	0.16
4.	0.020	2.0	0.16

Conclusion. The results indicate that aceto-acetic acid is not decomposed by the enzyme.

If the interfering chromogen is aceto-acetic acid then it ought to be possible to get rid of it by boiling the acidified blood filtrate with water.

Experiment 4. To 10.0 ml. of a 1 in 5 dilution 2.0 mg. per cent solution aceto-acetic acid was added 50.0 ml. water and one drop of dilute HCl (6N) and heated for 30 minutes on a hot-plate. The volume was then made up to 10.0 ml. with distilled water. 8.0 ml. of this solution was then taken and 4.0 ml. alkaline picrate added, and allowed to stand 10 minutes in the dark, at room temperature.

Conclusion. Preliminary experiments using aceto-acetic acid indicate that if aceto-acetic acid is the interfering chromogen then it is possible to get rid of it by boiling.

Experiment 5. 50.0 ml. of water was added to 10.0 ml. of a 1 in 5 serum filtrate, acidified with one drop of dilute acid (6 N) and boiled for 30 minutes on a hot plate. The solution was then cooled, the pH adjusted to 7.0, and the volume made up to 10.0 ml. with distilled water.

0.5 ml. phosphate buffer and 2.0 ml. of enzyme suspension were added and the solution incubated for 60 minutes. The solution was again cooled, and centrifuged at a high speed for 15 minutes.

To 8.0 ml. of the clear solution 4.0 ml. of alkaline picrate were added, and the solution allowed to stand in the dark, at room temperature, for 10 minutes. Readings were then taken on the S.P. 500 Spectrophotometer.

Results.

Serum No.	Scale Reading on S.P. 500	µg. in 8.0 ml. of filtrate	mg. per 100 ml. calculated as creatinine
1.	0.038	3.3	0.26
2.	0.032	2.8	0.22
3.	0.035	3.0	0.23
4.	0.035	3.0	0.23
Control (unheated)	0.028	2.4	0.19
Control (unheated)	0.028	2.4	0.19

Conclusion. The results would indicate that aceto-acetic acid is not the interfering chromogen, but since the interfering chromogen value tends to rise above the control value, there would appear to be some doubt as to the reliability of this experiment.

One of the most important properties of creatine and creatinine is the readiness of each, under appropriate conditions, to transform itself at least partially into the other. A creatine solution, if boiled with zinc, will yield upon cooling a precipitate of creatinine zinc chloride (Heintz, 1847; Dessaignes,

1857; Neubauer, 1861, I). If a creatine solution is treated with picric acid it will deposit in a short time crystals of creatinine picrate. Neubauer (1863, 1866) showed that when an aqueous solution of creatine is heated for three or four days in a closed tube immersed in boiling water, or simply evaporated slowly on a water-bath, the solute is converted, in the first case "almost completely", in the second "partly" into creatinine. Dessaignes (1857) observed that a slow conversion of creatinine into creatine occurred under the action of water alone, and Johnson (1888) was able, simply by boiling creatinine repeatedly with water, to convert all the creatinine into creatine. In fact the usual method of estimation of creatine is to convert it into creatinine by autoclaving the solution at 117°C for about 20 minutes in the presence of a sufficient concentration of hydrochloric acid.

In the face of this evidence it was difficult to believe how a purely chemical method could be developed for the determination of creatinine, by getting rid of the interfering chromogen by boiling the blood filtrate in an acid medium. Since it appeared more than likely that some, if not all, of the creatine would be converted into creatinine.

Experiment 6. 50.0 ml. of distilled water was added to 10.0 ml. of a 1 in 5 serum filtrate. One drop of dilute HCl was added and the solution heated on a hot-plate for 30 minutes.

The solution was then cooled, and the volume made up to 10.0 ml. with distilled water. 4.0 ml. of alkaline picrate was added to 8.0 ml. of the solution, and the colour measured in the S.P. 500 spectrophotometer. As a control, serum filtrates were prepared from the same serum and measured simultaneously with the boiled filtrates.

Results

No.		Scale Reading on S.P. 500	µg. creatinine in 8.0 ml. solution	mg. creatinine per 100 ml.
1.	Boiled with acid	0.220	19.4	1.21
2.	" " "	0.244	19.8	1.23
3.	" " "	0.220	19.4	1.21
4.	Control	0.182	16.1	1.08
5.	"	0.182	16.1	1.08
6.	"	0.180	16.0	1.00

Conclusion. The results show that when a serum filtrate is boiled with acid some of the serum creatine in the filtrate is converted into creatinine.

DISCUSSION.

As long ago as 1917, Hunter and Campbell showed that human erythrocytes contain a substance which is not creatinine, but which gives a typical Jaffé reaction. Later, Haymann, Johnston and Bender (1935) working with trichloracetic acid - serum filtrates, came to the conclusion that serum also contains a small amount of material, other than creatinine, which reacts with alkaline picrate. The work of Miller and Dubos (1937) has further confirmed the observations of these earlier workers, and their method for the determination of creatinine using specific enzymes, has in fact proved beyond any doubt, that there is in blood and plasma a chromogen other than creatinine which reacts with alkaline picrate. This constant finding in normal blood and plasma has been fully substantiated in the present investigation.

Despite the fact that it has been known for many years that some other normal constituent of the blood reacts with alkaline picrate, nobody, as yet, has succeeded in identifying this interfering chromogen. Several workers have made tentative suggestions as to what it might be, yet none of the suggestions stand the test of experiment, as is shown in Table 1 . Miller and Dubos (1937) did show, however, that glycocyamidine is not the interfering chromogen, by

the following evidence: (1) Experiments with the enzyme on serum filtrates and compared simultaneously with equivalent concentrations of pure glycocyamidine indicated that the decomposition of the chromogenic material in blood filtrates was very much quicker than the decomposition of a pure glycocyamidine solution. (2) The results obtained by a comparison of the alkaline picrate and dinitrobenzoic acid methods were almost identical, yet glycocyamidine is known to give a colour with alkaline picrate approximately three times the intensity, as that given with dinitrobenzoic acid. (Miller and Dubos, 1937).

In a study of persons with reduced renal function, Miller and Dubos (1937) found that the non-creatinine chromogen was increased in cases showing signs of uraemia. On the other hand, patients who were comparatively free of uraemic symptoms did not show any marked increase in the non-creatinine chromogenic material. They suggest that the increase in the non-creatinine chromogenic material may be related to the "phenol bodies" which are believed to be increased in the blood in uraemia. The work of Becker (1933) and confirmed by Harrison and Mason (1937) does seem to suggest that the signs of depression are due to the absorption of putrefactive products, especially phenols, from the intestinal tract. Unfortunately, phenol does not react with alkaline picrate; even other dihydric phenols, and dihydroxyphenylalanine, if present

in the blood to any extent, only react with alkaline picrate in a concentration beyond the normal physiological range. Of greater significance in the author's opinion is the accumulation of large quantities of fixed acids, e.g. β-hydroxybutyric acid and aceto-acetic acid ("uncompensated alkali deficit") which are produced in diabetic coma and, in particular, in the terminal stages of nephritis as a result of (1) impaired excretion of these acids, (2) insufficient intake of food, especially carbohydrates.

Experimental proof that aceto-acetic acid is the interfering chromogen has proved very difficult, despite supporting evidence from diabetic bloods containing 1.68, 4.68 and 1.15 mg./100 ml. of aceto-acetic acid (as creatinine), and three cases of uraemia containing 1.62, 1.24 and 1.15 mg./100 ml. of residual chromogen (as creatinine). Important evidence suggesting that the interfering chromogen is acidic, has been obtained from the following experiments. (1) It is known that the interfering chromogen is not adsorbed by kaolin or Lloyd's reagent; aceto-acetic acid is not adsorbed by these reagents. (2) The interfering chromogen is not decomposed by the enzyme.

Experiments with boiled serum filtrates in acid solution, always give a residual colour after treatment with the enzymes, and consequently this argues against aceto-acetic acid being the interfering chromogen. There are, however,

in serum filtrates some substances which on boiling with mineral acid form compounds which give a colour with alkaline picrate. For example, (i) Glucose forms a furfural compound which is known to react with alkaline picrate (Storey, 1912), and (ii) Creatine which is converted into creatinine and which also gives a colour with alkaline picrate.

Conclusive evidence as to the nature of the interfering chromogen is still lacking, yet, the evidence as reported here, incomplete though it may be, suggests that the interfering chromogen is acidic and may be aceto-acetic acid.

PART II. THE EXCRETION OF CREATININE BY THE HUMAN KIDNEY.

THE EXCRETION OF CREATININE BY THE HUMAN KIDNEY

INTRODUCTION

In putting forward his modern theory of renal function, Cushny (1926) pointed out that if any substance could be found which was eliminated entirely by glomerular filtration and was neither reabsorbed nor excreted by the cells of the renal tubules, then that substance could be employed to determine the actual rate of glomerular filtration. The truth of this statement can scarcely be questioned unless we suppose that the process occurring in the glomeruli is not one of simple filtration.

In 1926 Rehberg pointed out the apparent relative advantages of exogenous creatinine excretion as an index of filtration rate, and in more recent papers has published observations which he interprets as supporting the view that creatinine fulfils the necessary requirements, i.e., is filtered at the glomerulus at the same concentration as in the plasma, and is neither reabsorbed nor excreted by the tubular cells. Others, however, have claimed that, in man, creatinine is actively excreted by the tubular cells.

Of the many substances which have been tried and have stood the test of experiment, only inulin and creatinine have been used to any large extent as indices of glomerular filtration. Since 1935, however, inulin has been generally

accepted as the standard reference substance, but there is still some doubt as to its reliability. If the claims for both substances are to be justified it must be shown that the clearance results obtained by the use of creatinine are the same as those obtained with inulin, and that this agreement holds under all conditions, both physiological and pathological. The problem is further complicated by the fact that the results of Ferguson, Olbrich, Robson and Stewart (1949) strongly suggested that inulin is actively reabsorbed by the renal tubules. If the inulin clearance is not accepted as a measure of glomerular filtration rate the evidence for the tubular excretion of creatinine based upon a comparison of inulin and creatinine clearances is no longer valid. Under these circumstances the creatinine clearance conceivably measures the glomerular filtration rate, or it is possible that the glomerular filtration rate lies somewhere between the values obtained by creatinine and inulin clearance determinations.

The classical conception of renal clearance demands that if a substance is to be accepted as a true measure of glomerular filtration rate then the rate of excretion (UV) must vary directly as the plasma concentration (P) - i.e., $UV = \underline{k}P$. If, however, UV is independent of P or if $UV = \underline{k}P + \underline{x}$, then other mechanisms are at work, e.g. reabsorption or excretion, or both. Shannon (1935) showed

that when the plasma creatinine was raised to abnormally high levels by intravenous injection of creatinine, the creatinine clearances compared with simultaneous inulin clearances tended to drop as the creatinine concentration in the plasma was raised. Earlier technical difficulties in the accurate estimation of creatinine in the low concentrations encountered in the plasma of normal subjects, prevented the observations from being extended to include 'true' endogenous creatinine. Also in those days, the methods available for the estimation of inulin were crude and could only be used when the blood contained very high concentrations of inulin. It is possible that much of the confusion which still exists concerning the mode of excretion of creatinine could be attributable, in part, to inadequacies in many of the biochemical procedures of those days.

In the present investigation the results of a number of observations on the rate of excretion of "endogenous" and "exogenous" creatinine are presented. Despite the fact that both "endogenous" and "exogenous" creatinine are chemically identical, some uncertainty still remains as to how they are actually excreted. It was therefore decided to compare the creatinine clearances, at endogenous and at high and low plasma creatinine levels, with simultaneously determined inulin clearances, also at different plasma

inulin levels, and so decide whether creatinine is, or is not, a more accurate measure than inulin, of glomerular filtration rate.

ENDOGENOUS CREATININE CLEARANCE

Introduction

Miller and Winkler (1938) using the enzymatic method of Miller and Dubos (1937) for the estimation of true endogenous creatinine showed that in eight subjects without renal disease the 'true' endogenous creatinine/inulin ratios averaged 1.13 (range 0.9 to 1.5); in five of the eight experiments however the 'true' endogenous creatinine/inulin ratios averaged 0.99 (range 0.8 to 1.1). When they extended their investigation to include subjects with renal disease they found in four subjects that the endogenous creatinine /inulin ratios averaged 1.38 (range 0.9 to 1.7). These results differ from each other in one important respect, i.e., the endogenous creatinine clearance in the subjects with renal disease was regularly higher than the simultaneously determined inulin clearance, but in the normal subjects the endogenous creatinine clearance was in most cases the same as the inulin clearance. The conclusions drawn from this result were: (1) since the endogenous creatinine clearance in normal subjects equals the inulin clearance in many cases, then endogenous creatinine in normal subjects is a measure of glomerular filtration rate. (2) in subjects with renal damage the endogenous creatinine clearance was higher than the inulin clearance, and so in these cases

endogenous creatinine must be excreted by the tubules.

Brod and Sirota (1948) using a modified (1:4) Folin-Wu tungstate filtrate and the Bonsnes and Taussky creatinine method, showed that the endogenous creatinine/inulin clearance ratio in normal subjects averaged 1.00 (range 0.88 to 1.10). In 13 subjects with renal disease with a single exception the clearance ratios averaged 1.04 (0.89 to 1.25) and 1.10 (0.77 to 1.63). The discrepancy between the creatinine and inulin clearances of 10 per cent or greater appeared only in patients with filtration rates below 40 ml./min. The exception was a young female in the nephrotic stage of glomerulo-nephritis; in this instance endogenous creatinine was excreted in a manner similar to exogenous creatinine, the endogenous creatinine/inulin clearance ratio averaged 1.61. More recently Brod and Kotátko (1949) separated 'true' endogenous creatinine from the non-creatinine chromogen by adsorption of the former on Lloyd's reagent and obtained a total chromogen/inulin clearance ratio below 1.0 in several normal subjects. When the 'true' creatinine figure was substituted for the total "chromogen" figure the clearance ratio rose to 1.0 in all but one case. They also tried the effect of caronamide and high plasma levels of para-amino hippuric acid on endogenous creatinine excretion, but were unable to find any difference in the creatinine/inulin ratio.

Preliminary experiments now going on in this laboratory using caronamide would appear to substantiate the findings of Brod and Kotátko. Consequently they believe that 'true' endogenous creatinine is excreted without tubular reabsorption or excretion. Miller, Leaf, Mamby and Miller (1952) also using the enzymatic method of Miller and Dubos (1937) in a study of 9 pathological subjects (4 female and 5 male) were unable to confirm that the 'true' endogenous creatinine/inulin ratios were approximately 1.0, their figures corrected average 1.59 (range 0.59 to 1.64) for males, and corrected average 1.68 (range 0.82 to 1.74) for females.

Experimental

Source of Data. In all, six subjects had endogenous and exogenous creatinine clearances determined.

Experimental Procedure. All subjects fasted overnight and were recumbent during the experiment; no breakfast was given and a free flow of urine was established by allowing at least a pint of weak tea to be drunk at approximately 7 a.m. in the morning, followed by further cups of tea before the commencement of the experiment. A free flow of urine was maintained during the experiment by allowing further cups of weak tea and water to be drunk. The creatinine, in the case of the oral experiments was dissolved in very weak tea; when creatinine was injected intravenously it was dissolved in

isotonic saline and filtered through a Seitz filter. Venous and arterial blood were removed simultaneously at intervals of approximately ten to fifteen minutes; urine was always collected by catheter, except in two preliminary investigations, over periods of 10 to 15 minutes, the bladder being washed out with 10 ml. saline after each collection. The exact time noted for all operations.

Calculation of Clearances. For each period the renal clearance of creatinine was determined by dividing the mean rate of creatinine excretion per minute by the estimated mean plasma level of creatinine for the period. The latter was read from a smooth curve drawn through the plasma creatinine concentrations plotted against time. The concentration selected was one occurring 2½ minutes before the mid-point of the clearance period.

Results. The results in Table 1 show that when creatinine determinations are done under controlled conditions (i.e., time, temperature, and in the dark) and using a very sensitive spectrophotometer the 'true' endogenous creatinine clearance is invariably greater than the simultaneous inulin clearance, and the creatinine/inulin ratios are consistently greater than 1.0. If no allowance is made for the interfering chromogen then the apparent creatinine/inulin ratios are in two cases approximately 1.0, but in the majority of cases exceed 1.0 but are never below 1.0. This might explain

to some extent why in the past so many workers have got good agreement between their so-called endogenous creatinine and inulin clearance figures, and hence the assumption (on grounds of equality with the inulin clearance) that endogenous creatinine is a measure of glomerular filtration rate in man, always assuming that inulin is a true measure of glomerular filtration rate in man.

Discussion

Apart from the work of Miller and Dubos (1936), Miller and Winkler (1938) and Miller, Leaf, Mamby and Miller (1952) there appears to have been little or no other use made of the specific enzymatic method in renal clearance work. Adsorption techniques have been used to overcome the non-specificity of the Jaffé reaction, and have to some extent confirmed the results obtained by the enzymatic method. Despite these more accurate methods which are now available, controversy still continues as to whether the 'true' endogenous creatinine clearance is less than, equal to, or greater than the inulin clearance.

It has long been a matter of debate as to how much of the colour given by a plasma filtrate with alkaline picrate is attributable to creatinine, and insufficient consideration of this point has led in the past to uncertainty as to how endogenous creatinine is excreted by the kidneys. More important still is that if 'true' endogenous creatinine is

TABLE I.

A comparison of uncorrected and corrected creatinine clearances with inulin clearances

	Period No.	Cin	Cin Uncorrected Serum Creatinine mg/100 ml. (one determination)	Ccr Uncorrected	Mean Ccr / Cin	Corrected Serum Creatinine mg/100 ml. (one determination)	Ccr Corrected	Mean Corrected Ccr / Cin
R.B.	1.		0.80	125		0.65	147	
	2.		0.80	118		0.65	145	
	3.		0.80	110		0.65	141	
	4.	111	0.80	115	1.06	0.65	148	1.31
	5.		0.80	114		0.65	140	
	6.		0.80	123		0.65	148	
	7.		0.80	121		0.65	149	
J.A.O.	1.	108	0.92	112	1.05	0.74	140	1.31
	2.		0.92	115		0.74	143	
J.A.O.	1.	110	0.92	132	1.17	0.74	165	1.46
	2.		0.92	126		0.74	157	
W.	1.		1.20	84		1.04	97	
	2.	64	1.20	71	1.19	1.04	82	1.37
	3.		1.20	75		1.04	86	
W.H.	1.	91	1.09	106		0.87	133	
	2.	78	1.09	100	1.25	0.87	132	1.47
	3.	94	1.09	110		0.87	138	
	4.	77	1.09	105		0.87	132	
D.Oliver	1.	49	0.72	84	1.71	0.59	103	
	2.		0.72	78	1.59	0.59	96	
Huddert	1.	92	0.85	102	1.11	0.70	124	1.35
	2.	91	0.85	107	1.17	0.70	130	1.43

to be determined in the future with any degree of accuracy then a very sensitive instrument must be used, for it is quite impossible to obtain accurate and reliable results (more particularly of the non-creatinine chromogen) with many of the instruments which are in use at the present time. This might also be a partial explanation why Miller and Winkler (1938) and more recently Miller, Leaf, Mamby and Miller (1952) got many irregularities in their clearance ratios.

The important point which it is desired to stress at this stage, is that the 'true' endogenous creatinine clearance in the human subject, healthy or otherwise, is consistently higher than the inulin clearance. In no case has the inulin clearance ever approached the value for the creatinine clearance except in one patient (P.M., see Table III) with known tubular lesions when greater back diffusion is known to occur (Wearn and Richards, 1924), but this does not remain true if uncorrected creatinine clearances are considered. If inulin is reabsorbed to the extent of 15 per cent or more, (Ferguson, Robson, Olbrich and Stewart, 1949), and if 20 per cent of the apparent creatinine in a plasma filtrate is due to the interfering chromogen, then the two errors will tend to cancel each other out and so both methods, inulin and creatinine, will give comparable figures for the "rate of glomerular filtration."

EXOGENOUS CREATININE CLEARANCE

Introduction

Many workers have shown, in man, that the exogenous creatinine/inulin ratios after ingestion or single intravenous injection of creatinine average approximately 1.4 (Shannon, 1935; McCance and Widdowson, 1937; Miller and Winkler, 1938; Shannon and Ranges, 1941; Josephson and Godin, 1943; Dean and McCance, 1947; Odell, 1947; Brod and Sirota, 1948); and an average ratio of 1.25, after a continuous infusion of creatinine is also reported by Crawford (1948). An average clearance ratio of 1.4, in man, would indicate that about 28 per cent of the total creatinine in the urine is excreted by the tubules, on the assumption that inulin is a true measure of glomerular filtration rate. If, however, approximately 20 per cent of the filtered inulin is reabsorbed, then exogenous creatinine clearance might also be a 'true' measure of glomerular filtration rate.

The alleged excretion of creatinine by the tubules would appear to be further complicated, since after the ingestion of creatinine the clearances have a real tendency to rise erratically above the 'true' endogenous clearance. This could mean that endogenous and exogenous creatinine are excreted by different mechanisms, yet there does not appear to be any logical reason why this should be the case, since a molecule

of creatinine, whether it be endogenous or exogenous, is still a molecule of creatinine chemically, and physiologically. The author's results show this phenomenon quite well, and leave no doubt that the exogenous clearances following the ingestion of creatinine are consistently higher than the 'true' endogenous clearances, thus confirming the results of the above workers.

At this stage it seemed just possible that creatinine, when given by mouth or by a single intravenous injection, might produce different concentrations in the arterial and venous blood, and that this might be the cause of the apparently varying relationship between the plasma concentration of creatinine (P) and the amount excreted per minute (UV).

INVESTIGATION OF THE ARTERIO-VENOUS DIFFERENCE.

When Rehberg (1926) suggested the use of exogenous creatinine as a measure of glomerular filtration rate he was very careful to emphasise the use of arterial blood in all renal clearance work, since the kidney filtered arterial blood. Since Rehberg's time, however, practically all renal clearance work has usually been done with venous blood, mainly because it is easier to obtain and the patient is not submitted to the same discomfort or danger. If, however, more attention had been paid to this remark by Rehberg, then it is quite possible that much of the confusion which still exists in the field of exogenous creatinine clearance work, especially after ingestion of creatinine, would not have existed.

Brun, Hilden and Raaschou (1949) made a detailed study of arterial and venous differences and found that the venous blood (on a falling plasma curve) was 28.7 per cent higher in diodone and 7.4 per cent higher in inulin than arterial blood withdrawn at the same time. On a rising plasma curve the error will be of the same magnitude but opposite in direction, and the calculated clearances will be much too high. The method of intravenous infusion ensures that the arterial and venous concentrations of creatinine are equal (Brun, Hilden and Raaschou, 1949) since the rate of infusion

is normally adjusted to approximately the rate of excretion, always provided that the plasma concentration and the volume of distribution are constant. When, however, creatinine was taken by mouth or was injected intravenously it appeared likely that this state of affairs no longer held, and might explain the apparent rise in the creatinine clearances on a rising plasma curve. It is well established that significant errors are introduced in clearance determinations by any method where the plasma concentration of the reference substance is changing rapidly for one or more of the following reasons:- (1) the volume of distribution is not constant, (2) there is unequal distribution of the substance, (3) the rate of clearance into the bladder is not constant.

Experimental. The subjects were prepared as described in the preceding section. Starting about 45 minutes after ingestion, simultaneous arterial and venous blood samples were obtained at intervals of about 15 minutes. Venous blood was obtained from a vein in the cubital fossa, and arterial blood was withdrawn from the femoral artery just below the inguinal ligament. All operations were accurately timed.

Results. Since, in spite of all precautions, some venous and arterial blood samples were obtained at slightly different times in the same subject, direct comparison of the two

concentrations was not always possible. Accordingly, all venous concentrations were plotted against time, and a smooth curve constructed through the points by visual approximation. The arterial concentrations were then plotted on the same graph, and were compared with the venous serum concentrations read from the smooth curve at the times of the arterial estimations.

The results and the arterio-venous differences at the times of the arterial estimations are given in Table II.

The average arterio-venous difference for each individual with a rising plasma curve varies from + 10 per cent to + 20 per cent. On a falling curve the difference varies from + 0.5 per cent to - 1 per cent. Estimates of the renal clearance of creatinine in these subjects are also given in Table III.

Discussion. The discussion of the results will be confined to the relation they have to the phenomenon of the rise or fall in the renal clearance of creatinine following the ingestion or injection of creatinine. It is clear that any absolute difference between the concentrations of creatinine in the venous plasma and in the arterial plasma will affect the value for clearance by the magnitude of that difference. A few arterio-venous differences were determined on endogenous serum creatinine, and the difference would appear to be within the limits of the method. The results do show, however, a

TABLE II

The concentration of creatinine in arterial and venous blood.
Time, t, indicates the time in minutes following creatinine given orally and intravenously.

Subject	Sex	Age Years	C cr	Time t	P Venous	P Arterial	A-V difference	% of venous	
McG	M	56	114	77	9.62	9.94	0.32	3	
				100	9.72	9.75	0.03	0.3	
				Average	percentage	difference			2
McR	M	-	167	49.5	5.87	6.65	0.78	13	
				64	7.16	7.74	0.58	8	
				Average	percentage	difference			10
P.M.	M	-	58	42.5	7.06	8.62	0.56	8	
				57	10.44	12.19	1.75	17	
				74	13.69	15.81	2.12	15	
				Average	percentage	difference			20
C.M.	M	-	97	27	1.08	1.08	0	0	
				43	69.75	70.25	0.5	1	
				59	65.31	65.31	0	0	
					62.00	62.00	0	0	
				Average	percentage	difference			0.5
D.	M	25	138	22	62.30	63.00	0.70	1	
				43	53.30	50.60	0.30	0.5	
				61	45.50	44.00	- 1.50	-3	
				Average	percentage	difference			-1

marked difference on a rising plasma curve, using venous blood and will account for the clearances being too high, with a falling plasma curve the differences are almost beyond the precision of the estimation. It may be concluded, therefore, that differences between the venous and arterial concentration of creatinine, following creatinine, do explain the apparent rise in the clearance of creatinine with rising plasma concentrations, with falling plasma concentrations the differences, within the range studied, would not appear to explain the slight fall in the creatinine clearance. Of course, it is possible that as the plasma concentration falls to extremely low values, then the arterio-venous difference might become significant.

TABLE III.

R. Baillie, age 26 years.

Period No.	C In. ml/min.	Urine Volume	Time	Dilution	Reading	ug/8 ml.	mg/100 ml.	Total UV mg/period	uv/min. mg/min.	Pc uncorrected	Pc corrected	Cor. corrected	Cor. uncorrected
Endogenous crestinine													
1.		346	28	1/50	0.130	11.1	6.95	24.00	0.86	0.80	0.65	132	107
2.	108	275	22	1/50	0.134	11.3	7.06	19.40	0.88	0.80	0.65	135.5	110
3.		112	32	1/100	0.256	20.8	26.0	29.14	0.91	0.80	0.65	140	114
5.0 g. crestinine given orally													
4.		206	32	1/200	0.117	9.8	24.50	50.57	1.56		1.10	142	
5.													
6.		472	26	1/200	0.205	17.3	43.40	204.8	7.9		4.52	174	
7.		377	19	1/200	0.185	15.7	39.30	148.2	7.8		4.92	158	
2.5 g. crestinine given orally													
8.													
9.		242	34	1/1000	0.230	19.2	240.0	580.8	17.1		10.35	165	

Webster

Period No.	C In. ml/min.	Urine Volume	Time	Dilution	Reading	ug/8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Po uncorrected	Po corrected	Cor. corrected	Cor. uncorrected
Endogenous Creatinine													
1.													
2.		65	28	1/200	0.220	17.4	43.5	28.27	1.00	1.200	1.04	97.5	84.5
3.	64	217	34	1/200	0.072	5.7	14.25	30.92	0.91	1.20	1.04	87.5	76
4.		165	22	1/200	0.065	5.0	12.50	20.62	0.94	1.20	1.04	90.0	78.5
5.0 g. creatinine given orally													
8.													
9.		130	45	1/2000	0.159	12.6	315.0	409.50	9.10		7.62	119.0	

John McCaig, age 52 years.

Period No.	C In. ml/min.	Urine Volume	Time	Dilution	Reading	ug./8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Pc uncorrected	Pc corrected	Cor. corrected	Cor. uncorrected
Endogenous creatinine.													
1.		44	27	1/500	0.134	11.0	68.75	30.25	1.12	1.01	0.82	137	111
2.		67	24	1/200	0.182	15.0	37.50	25.12	1.04	1.01	0.82	128	104
3.	80	54	11	1/100	0.210	17.2	21.50	11.61	1.05	1.01	0.82	129	100
4.		84	11	1/100	0.136	11.2	14.0	11.76	1.07	1.01	0.82	130	106
5.		115	12	1/100	0.110	9.1	11.35	13.05	1.09	1.01	0.82	133	108
5.0 g. creatinine given orally													
6.		130	12	1/500	0.198	16.2	101.25	131.62	10.97	-	7.4	148	
7.		166	15	1/500	0.235	19.4	121.25	201.27	13.41	-	8.8	153	
8.		139	12	1/500	0.250	20.6	128.5	178.61	14.88	-	10.15	147	
9.		124	11	1/500	0.267	24.5	137.5	170.50	15.50	-	11.15	139	

Patrick Murphy, age 64 years.

Period No.	C In. ml./min.	Urine Volume	Time	Dilution	Reading	ug./8 ml.	mg./100 ml.	Total UV mg./min.	uv/min. mg./min.	Pc uncorrected	V)))) ▲) Pc corrected		Cor. corrected	Cor. uncorrected
Endogenous Creatinine														
1.														
2.		110	12	1/100	0.108	9.1	10.40	11.41	0.95	1.70	1.55	-	61.5	56
3.	45	110	12.75	1/100	0.108	8.3	10.40	11.41	0.89	1.70	1.55	-	58	53
4.		135	26	1/100	0.172	13.2	16.50	22.27	0.85	1.70	1.55	-	55	50.5
10.0 g. creatinine given orally													Cor.	
												8.62	51	
5.		125	17	1/500	0.125	9.6	60.00	75.00	4.41		7.06		63	
												12.19	54	
6.		66	16	1/2000	0.082	6.35	158.75	104.74	6.45		10.44		63	
												15.81	59	
7.		53	15.25	1/2000	0.142	10.9	270.0	143.00	9.37		13.69		68	

McGill, age 56 years

Period No.	C In. ml./min.	Urine Volume	Time	Dilution	Reading	ug./8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Po uncorrected	Pc corrected (V) (▲)		Cor. corrected	Cor. uncorrected
Endogenous creatinine														
1.		118	16	1/50	0.218	16.25	10.16	11.99	0.75	0.79	0.61		123	95
2.		93	12	1/50	0.188	14.0	8.75	8.14	0.68	0.79	0.61		111	86
3.	72	72	12.25	1/50	0.247	18.4	11.50	8.28	0.67	0.79	0.61		111	86
4.		61	11.25	1/50	0.272	20.4	12.75	7.78	0.69	0.79	0.61		113	86
5.0 g. creatinine given orally in 200 ml. of weak tea.													Cor.	
5.		195	43	1/1000	0.277	20.6	257.5	502.12	11.63		9.81	10.25	113	
													119	
												9.94	115	
6.		125	19	1/1000	0.186	13.9	173.75	217.19	11.43		9.62		119	
												9.75	106	
7.		120	20	1/1000	0.183	13.7	171.25	205.50	10.27		9.72		106	

McRobbie.

Period No.	C In. ml./min.	Urine Volume	Time	Dilution	Reading	ug./8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Pc uncorrected	V) Pc corrected A)		Cor. corrected	Cor. uncorrected
Endogenous creatinine														
1.		171	15.5	1/50	0.220	17.7	11.05	18.89	1.21	0.85	0.70	-	174	143
2.	133	212	11	1/50	0.114	9.3	5.81	12.31	1.11	0.85	0.70	-	160	132
3.		222	10.5	1/50	0.110	9.0	5.62	12.47	1.18	0.85	0.70	-	170	139
4.		165	8.0	1/50	0.110	9.0	5.62	9.27	1.15	0.85	0.70	-	165	136
5.0 creatinine given orally													Cor.	
5.		80	15.0	1/1000	0.197	15.9	198.75	159.0	10.60	-	5.87	6.65	160	
6.		46	12.75	1/2000	0.186	15.0	375.0	172.50	13.53	-	7.16	7.74	180 175 189	

OBSERVATIONS ON THE RENAL CLEARANCE OF CREATININE EMPLOYING CONTINUOUS INFUSION

Introduction.

Numerous workers have commented on the relative constancy of the excretion rate of creatinine over a period of time, and have shown that it is unaffected by change in the urine volume and by water diuresis or diuretic agents (van Hoogenhuyze and Verploegh, 1905; Klercker, 1907; Shaffer, 1908). Rehberg (1926) found, however, that under ordinary circumstances a constant excretion level of creatinine in the plasma does not lead to a constant excretion rate of creatinine in the urine. There is now, however, ample evidence to indicate that these findings of Rehberg were incorrect for Mackay (1929-30) has shown in one individual that under controlled conditions in man, the rate of excretion of creatinine is directly proportional to the plasma concentration up to 15 mg. per 100 ml; and Cope (1931) obtained a similar result in another individual up to 8 mg. per 100 ml; and Jolliffe and Chasis (1933) who worked between plasma concentrations of 2.4 to 11.6 mg. per 100 ml. in one instance, and 1.8 to 7 mg. per 100 ml. in a second, also Findlay (1938) who worked between plasma levels of 1.6 to 13.8 mg. per 100 ml., and Shannon and Ranges (1941) found in one normal subject, using a constant infusion of creatinine at plasma levels of 10.3 to 13.85 mg. per 100 ml., that the average of three clearance

periods at each of three different levels of creatinine were 193, 182 and 170. The results of these workers who all used creatinine which had been given by single intravenous injection or by mouth, except Shannon and Ranges, strongly suggest that in man the rate of excretion is directly proportional to the plasma concentration up to approximately 14 mg. per 100 ml., so that if UV were plotted graphically against P the result would be a straight line passing through the origin, indicating that creatinine is neither excreted nor reabsorbed by the tubules and is therefore a measure of glomerular filtration rate in man, at these plasma concentrations of creatinine. If, however, creatinine is to be a true measure of the glomerular filtration rate then it must be shown that at both high and low plasma levels the same proportionality exists between UV and P, and that this line does go through the origin.

With the aid of inulin, introduced by Smith and Shannon (1935) as a measure of the glomerular filtration rate in man, Shannon (1935) investigated the excretion of creatinine at high plasma levels, since there still existed some doubt regarding the original belief held by Rehberg that exogenous creatinine could be used as a measure of glomerular filtration rate in man. At low concentrations of creatinine (7.3 to 13 mg. per 100 ml.) Shannon found that the creatinine clearance was 30 to 45 per cent higher than the inulin clearance, the mean

value being 1.39. As the plasma concentration of creatinine was raised so the creatinine clearances fell absolutely and relatively to the inulin clearance, until at plasma concentrations of 96 to 127 mg. per 100 ml. the creatinine clearance was only 12 per cent higher than the inulin clearance. Shannon, interpreted his results as indicative of a curvilinear relationshop between the rate of excretion and high plasma concentrations, as against a proportional relationship between the rate of excretion and low plasma concentrations found by Mackay, Cope, Findlay, and Jolliffe and Chasis. Shannon, therefore, came to the conclusion that, because the clearances decrease as the plasma concentration increases, then excretion by the tubules must be involved in the excretion of creatinine.

Experimental. Four volunteers were used for the experiments, and as far as was known were all in fairly good health.

In all four subjects the type of experiment was the same, consecutive clearances being determined at each of four levels of plasma creatinine (endogenous and three exogenous levels). A primary injection of 50 ml. of 5.0 per cent creatinine in isotonic saline was given, followed by a sustaining infusion of creatinine (1.5 g. per cent at 1 ml/min.). After about 30 minutes four creatinine clearances were determined; a second primary injection was then given

(250 ml. of 6 per cent), followed by a sustaining infusion of creatinine (3.8 g. per cent at 2 ml./min.). After about 30 minutes another four clearances were determined. The procedure was repeated following a third primary injection (250 ml. of 6 per cent), followed by a sustaining infusion of creatinine (3.8 per cent at 4 ml./min.).

In the case of W.H. whose clearances were also determined at low plasma levels of creatinine, a primary injection of 25 ml. of 3 per cent creatinine in isotonic saline was given, followed by a sustaining infusion of creatinine (0.6 g. per cent at 1 ml./min.). After allowing 30 minutes for equilibration, four clearances were determined, a second primary injection was then given (25 ml. of 3 per cent) followed by a sustaining infusion of creatinine (0.6 per cent at 2-3 ml./min.). After about 30 minutes another four clearances were determined. The procedure was repeated following a third priming injection (25 ml. of 3 per cent) followed by a sustaining infusion of creatinine (0.6 per cent at 4 ml./min.).

The creatinine and inulin were pure products and the solutions were made up in isotonic saline and filtered through a Seitz filter prior to injection. In all the infusions the rate was that calculated to give a constant plasma creatinine level. Inulin does not react with alkaline picrate, at the concentrations studied, nor does

creatinine interfere with the Seliwanoff reaction which was used for the determination of inulin.

Clearance periods were of 10 to 20 minutes duration, and venous blood samples were withdrawn 2 minutes before the beginning and 2 minutes before the end of each period. The mean plasma concentration of these two samples was employed in the calculation of clearance for the period. The bladder was washed out with 10 ml. of normal saline at the end of each period. All operations were timed, and creatinine was determined in all samples by the method already indicated in an earlier section of this thesis, except that a new calibration curve was always prepared on the day that the estimations were determined. The preparation of the subject was as described in preceding sections. All the subjects were recumbent and kept warm during the experiment.

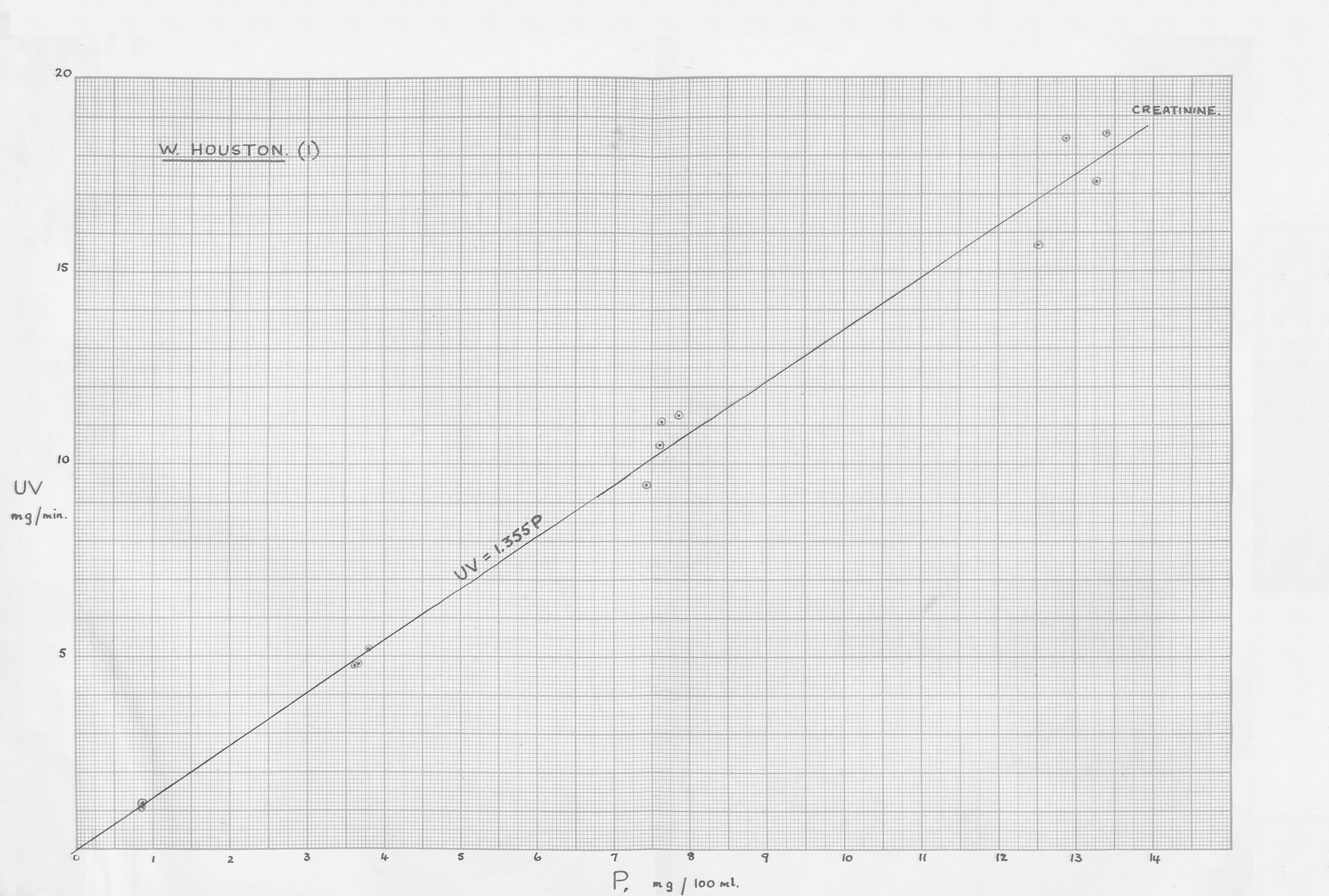
Results. The results are given in Table IV together with the graphs, when UV was plotted against P. The graphs illustrate the observed relationship between the concentration of endogenous and exogenous creatinine in the serum and its rate of excretion. There is some scattering of the points, but in no case is there any doubt as to the general direction of the line, even by visual approximation, including endogenous levels of plasma concentration, provided due consideration is taken and an allowance made for the non-creatinine chromogen fraction, determined by the enzymatic method

TABLE IV

W. Houston (1)

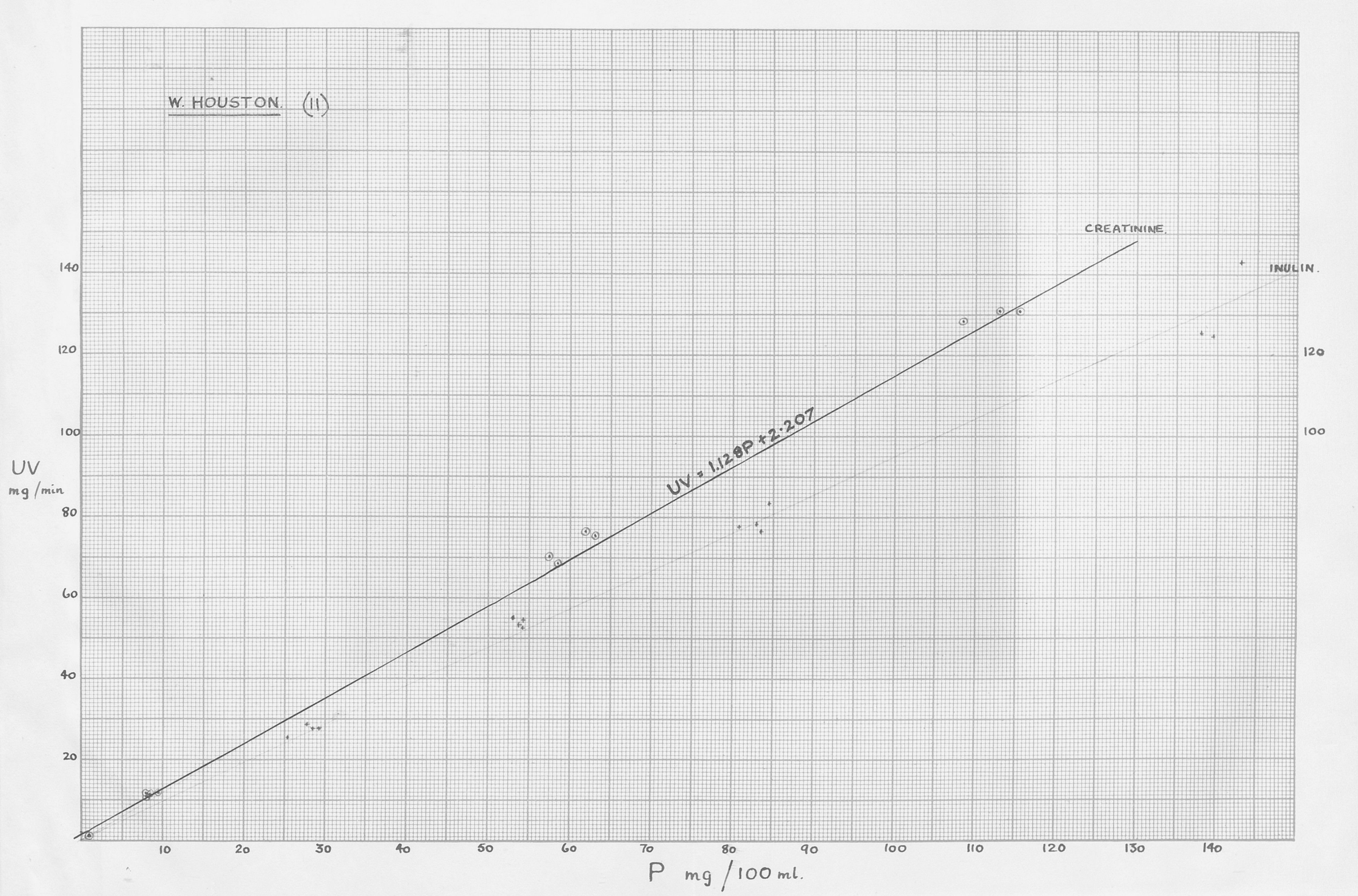
Period No.	C In.	Mean C In.	Volume	Time	Dilution	Reading	µg./8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Pc mg./100 ml.	Cor.	Mean COr.
Endogenous creatinine													
1.	91		76	9.0	1/200	0.067	5.5	13.75	10.45	1.16	0.87	133	
2.	78	85	73	10.5	1/200	0.078	6.6	16.50	12.04	1.09	0.87	125	132
3.	94		95	12.25	1/200	0.075	6.2	15.50	14.72	1.20	0.87	138	
4.	77		108	14	1/200	0.072	6.0	15.00	16.20	1.15	0.87	132	
Exogenous creatinine													
1.	80		105	12	1/500	0.115	9.5	59.375	62.36	5.2	⁂3.80	137	
2.	80	78	84	13	1/500	0.143	11.9	74.375	62.48	4.8	⁂3.67	131	133
3.	73		80	14.5	1/500	0.166	13.8	86.25	69.00	4.75	⁂3.62	131	
4.													
1.	73		67	11.5	1/1000	0.157	13.5	162.5	108.9	9.47	7.43	138	
2.	79	84	114	15.5	1/1000	0.137	11.4	142.5	162.5	10.48	7.62	137	138
3.	98		78	8.0	1/1000	0.110	9.1	113.75	88.8	11.10	7.65	145	
4.	88		90	9.0	1/1000	0.108	9.0	112.5	101.25	11.25	7.87	143	
1.	78		121	12.5	1/1000	0.155	14.5	162.5	196.6	15.73	12.50	126	
2.	101	91	111	10.0	1/1000	0.160	13.3	166.25	184.5	18.45	12.87	143	134
3.	93		97	9.25	1/1000	0.160	13.3	166.25	161.4	17.33	13.27	132	
4.	94		102	10.5	1/1000	0.182	15.1	188.75	192.5	18.57	13.40	137	

⁂ Correction of 0.2 for interfering chromogen.


W. HOUSTON. (1)
CREATININE.
UV = 1.355P
UV
mg/min.
P, mg / 100 ml.
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
5
10
15
20

W. Houston (II)

Period No.	C In.	Mean C In.	Urine Volume	Time	Dilution	Reading	µg./8 ml.	mg./100 ml.	Total UV mg./period	uv/min. mg./min.	Pc mg./100 ml.	Ccr.	Mean Ccr.
Endogenous creatinine													
1.	95		48.5	14.5	1/100	0.306	23.2	29.0	14.05	0.97	0.85	116	
2.	96		47	12.0	1/100	0.255	19.5	24.4	11.35	0.93	0.85	112	
3.	103	99	51	11.0	1/100	0.230	17.6	22.0	11.20	1.02	0.85	120	115
4.	101		50	10.75	1/100	0.210	16.0	20.0	10.00	0.93	0.85	112	
Exogenous creatinine													
1.	102		94	11.5	1/1000	0.154	11.7	146.25	137.50	11.95	9.41	127	
2.	101		142	15.25	1/1000	0.131	10.0	125.0	177.50	11.65	8.59	136	
3.	93	99	92.5	10.75	1/1000	0.130	9.9	123.5	114.00	10.60	8.22	129	130
4.	100		130	12.75	1/1000	0.120	9.1	113.5	147.50	11.55	8.12	143	
1.	96		153	8.5	1/5000	0.090	6.8	425.0	650.0	76.5	62.0	123	
2.	95		151	8.75	1/5000	0.092	7.0	437.5	660.0	75.5	62.87	120	
3.	98	96	188	12.5	1/5000	0.095	7.3	456.25	877.5	68.75	58.62	117	120
4.	99		153	9.5	1/5000	0.092	7.0	437.5	670.0	70.50	57.37	123	
1.	89		320	22.0	1/10000	0.095	7.2	900.0	2888.0	131.0	115.5	113	
2.	100	93	117	9.0	1/10000	0.106	8.1	1012.5	1185.0	132.0	113.0	117	116
3.													
4.	91		119	11.0	1/10000	0.125	9.5	1185.0	1410.0	128.5	108.45	118	


W. HOUSTON. (11)
CREATININE.
INULIN.
UV = 1.128P + 2.207
UV
mg / min
P mg / 100 ml.
20
40
60
80
100
120
140
10
20
30
40
50
60
70
80
90
100
110
120
130
140

Urine

Period No.	Cin	Mean Cin	Volume	Time	Dilution	Reading	µg/8 ml.	mg/100ml.	Total UV mg./period	uv/min. mg./min.	Pc mg./100 ml.	Ccr.	Mean Ccr.
Endogenous Creatinine													
1.	106		70	17	1/100	.205	16.3	20.37	14.26	0.84	0.59	142	
2.	108	108	74	13	1/100	.153	12.1	15.12	11.19	0.86	0.59	146	
3.	109		93	13	1/100	.120	9.5	11.87	11.04	0.85	0.59	144	144
4.	109		79	12.5	1/100	.137	10.9	13.62	10.76	0.86	0.59	145	
Exogenous Creatinine													
1.	118		106	10	1/1000	.118	9.4	117.5	124.55	12.45	8.7	143	
2.	105	111	70	14.5	1/1000	.260	20.6	257.5	180.25	12.42	8.8	141	143
3.	101		88	18	1/1000	.268	21.2	265.0	233.20	12.95	8.95	145	
1.	106		105	10	1/5000	.140	11.1	693.75	728.43	72.84	51.4	142	
2.	110		76	14	1/5000	.222	17.7	1106.25	840.75	60.0	43.0	140	
3.	107	107	52	11.25	1/5000	.276	22.0	1375	715.0	63.55	44.5	143	141
4.	103		58	14	1/5000	.318	25.2	1575	913.5	65.25	46.00	140	
1.	123		152	11.5	1/10000	.122	9.7	1212.5	1843.0	160.5	107.25	149	
2.	125	136	126	9	1/10000	.124	9.8	1225	1543.5	171.75	116.25	147	147
3.	148		106	7.5	1/10000	.128	10.2	1275	1351.5	180.0	122.5	147	
4.	120		140	10.5	1/10000	.136	16.8	1350	1890.0	180.0	125	144	

J. CONWAY.

CREATININE.

INULIN.

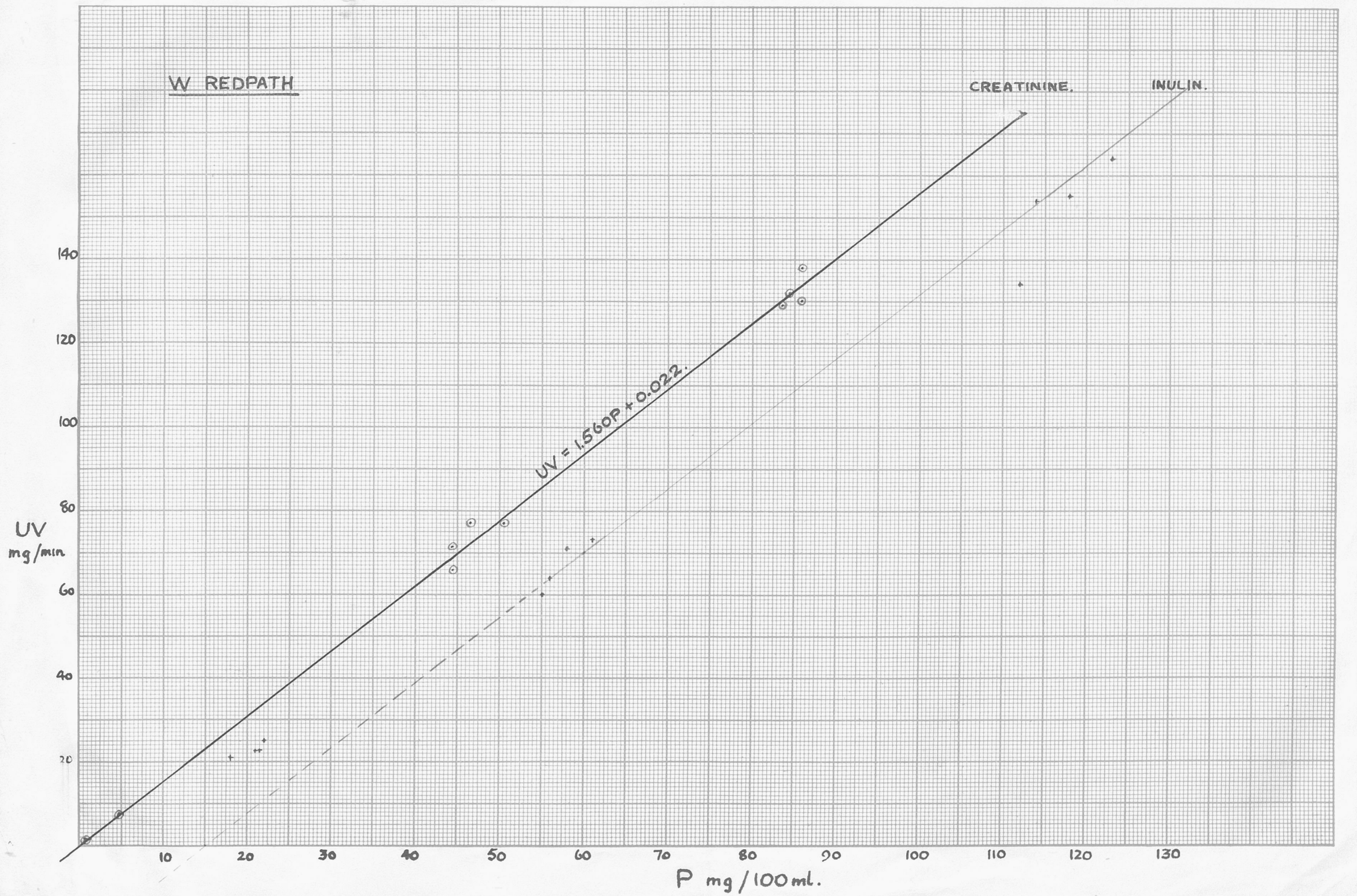
$UV = 1.407P + 2.051$

UV
mg / min.

P mg / 100 ml.

Period No.	Cin	Mean Cin	Urine Volume	Time	Dilution	Reading	µg/8 ml.	mg/100 ml.	Total UV mg./period	uv/min. mg./min	Pc mg./100 ml.	Ccr.	Mean Ccr.
Endogenous creatinine													
1.	94		34	12.25	1/200	.197	16.4	41.0	13.95	1.13	0.74	153	
2.	95		54	12.5	1/200	.125	10.4	26.0	14.04	1.12	0.74	152	
3.	-	101	128	15.5	1/200	.067	5.5	13.75	17.60	1.13	0.74	153	152
4.	103		131	9	1/100	.074	6.1	7.62	9.98	1.11	0.74	150	
5.	113		159	9	1/100	.064	5.3	6.62	10.00	1.11	0.74	150	
Exogenous creatinine													
1.	121		115	9.75	1/500	.126	10.5	65.62	75.03	7.69	4.95	155	
2.	106	106	128	13.50	1/500	.154	12.8	80.0	102.4	7.58	4.95	153	155
3.	114		107	11.75	1/500	.170	14.0	87.5	93.62	7.96	4.95	160	
4.	105		80	11.25	1/1000	.103	8.5	106.25	85.00	7.55	4.97	152	
1.	120		72	12.25	1/10000	.082	10.5	1312.5	945.0	77.14	50.62	152	
2.	121	115	70	13.25	1/10000	.090	11.7	1462.5	1023.75	77.26	46.56	165	156
3.	113		62	13	1/10000	.093	12.0	1500.0	930	71.53	44.37	161	
4.	112		50	12	1/10000	.096	12.6	1575.0	787.5	65.62	44.56	147	
1.	137		113	11.5	1/10000	.082	10.5	1312.5	1483.12	128.9	83.75	154	
2.	132	130	111	11	1/10000	.082	10.5	1312.5	1456.8	132.44	84.37	157	156
3.	138		129	11.5	1/10000	.082	10.5	1312.5	1593.12	138.53	86.25	160	
4.	120		65	6.5	1/10000	.082	10.5	1312.5	853.12	131.25	86.25	152	

W REDPATH


CREATININE.
INULIN.
UV = 1.560P + 0.022.
UV
mg/min
140
120
100
80
60
40
20
10
20
30
40
50
60
70
80
90
100
110
120
130
P mg/100ml.

Charles Muirhead, age 76 years

Period No.	C.in ml/min.	Volume	Time	Dilution	Reading	Mg/8 ml.	mg/100 ml.	Total UV mg./ period	uv/min. mg/min	v Pc uncorrected	V)) Pc corrected A)		Cor corrected	Cor uncorrected
Endogenous creatinine														
1.	71.5	48	19.25	1/200	.190	15.20	38.0	18.24	.94	1.08	0.90	0.90	105	87.7
2.	82.0	36	18.5	1/200	.205	16.45	41.12	14.8	.80	1.08	0.90	0.90	89	74
3.														
4.	91.5	51	13.25	1/200	.110	8.85	22.12	11.28	.85	1.08	0.90	0.90	95	79
40.0 g. creatinine in 500 ml. saline injected intravenously.														
5.	80.1	96.5	14.25	1/10000	.141	11.3	1412.5	1363.1	95.65		99.31		96	
6.	75.6	66.5	12.75	1/10000	.165	13.25	1656.25	1035.15	81.19		93.75		93	
7.	66.5	53	14	1/10000	.203	16.3	2037.5	1079.88	77.13		89.75		85	
8.	75	97.5	26.5	1/10000	.212	17.0	2125.0	2071.88	78.18		84.37		92.5	
Infusion stopped and the plasma creatinine level allowed to fall														
9.	74	42	14.5	1/10000	.205	16.45	2056.25	863.62	59.56		69.75	70.25	85.5	85
10.	63.5	38.5	16.0	1/10000	.192	15.4	1925.0	714.12	44.63		65.31	65.31	72	72
11.	76	34.5	15.5	1/10000	.218	17.5	2187.5	754.69	48.69		62.00	62.00	80	80
12.	61.7	32	16	1/10000	.220	17.65	2206.25	706	44.12		59.68		74	

* Urine collecting?

MUIRHEAD

CREATININE.

INULIN.

$UV = 0.905P + 0.034.$

UV
mg / min.

140
120
100
80
60
40
20

10 20 30 40 50 60 70 80 90 100 110 120 130.

P. mg / 100 ml.

Denholm, age 25 years.

Period No.	Volume	Time	Dilution	Reading	µg/8 ml.	mg/100 ml.	Total UV/period	uv/min.mg./min.	Pc uncorrected	V) Pc corrected A)	Cor corrected	Cor uncorrected
Endogenous creatinine												
1.	340	133	1/500	.108	8.65	54.0	183.6	1.38	1.16	0.98	141	119
30.0g. creatinine in 500 ml. Isotonic saline injected intravenously.												
2.	87	10.8	1/10000	.116	9.25	1153	980.2	92.87		65.4	151	
3.	131	14	1/5000	.190	15.2	952.5	1247	89.13		61.6	144	
4.	62	7.8	1/10000	.094	7.5	940	582.8	74.71		64.4	116	
Infusion stopped and serum creatinine allowed to fall										63.0		131
5.	98	22	1/20000	.092	7.4	1850	1702	77.38		62.3	132	
6.	81	21	1/20000	.079	6.3	1580	1280	60.95		55.3	114	120
7.	75	18.3	1/20000	.080	6.4	1610	1206	65.98		45.5	145	150
										50.6		
										44.0		

TABLE V.

Data of Shannon (1935). Results of creatinine clearance obtained using infusion of creatinine at different levels of plasma creatinine concentration.

J.C.

Period No.	Duration	Urine Flow	Plasma		Clearance		Clearance ratios
			Inulin	Creatinine	Inulin	Creatinine	Creatinine / Inulin
	min.	cc. per min.	mg.%	mg.%	cc. per min	cc. per min	
		After 15 g. creatinine by mouth					
1.	13	4.23	289	9.7	107.5	146.5	1.36
2.	11	4.25	249	10.1	113.0	153.0	1.35
3.	13	4.77	220	10.6	115.0	156.5	1.36
		After 10. g. creatinine intravenously					
4.	12	6.41	149	50.0	112.0	144.0	1.28
5.	12	4.82	128	48.8	108.5	138.5	1.28
6.	11	4.55	114	37.9	116.0	134.0	1.26
		After 30 g. creatinine intravenously					
7.	12	8.66	268	111.0	105.0	119.0	1.13
8.	13	7.82	226	91.3	119.0	136.0	1.13
9.	11	6.1	189	81.3	111.0	127.0	1.15

TABLE V.

Data of Shannon (1935). Results of creatinine clearance obtained using infusion of creatinine at different levels of plasma creatinine concentration.

	W.F.			S.C.		
	Number of observations	Plasma	Average Clearance	Number of observations	Plasma	Average Clearance
		m.gm. per cent	cc. per minute		m.gm. per cent	cc. per minute
Inulin	2	> 400	80	6	> 300	114
	7	301 - 400	76	8	201 - 300	109
	10	201 - 300	75	7	101 - 200	97
	11	< 201	77	3	< 101	109
Creatinine	5	> 91	80	3	> 99	121
	6	71 - 90	90	6	71 - 99	116
	7	41 - 70	91	5	41 - 70	127
	6	21 - 40	105	7	12 - 40	143
	6	1 - 10	112	3	10 - 11	164

of Miller and Dubos (1937). If no allowance is made for the interfering chromogen the line might not tend to go through the intersection of the axes, but might cut the base line, suggesting tubular reabsorption but not excretion.

The results show quite definitely that the clearance of endogenous creatinine becomes identical with the exogenous clearance, only when the former is corrected for that fraction of plasma chromogen which is not creatinine. Preliminary experiments have also shown that very little, if any, of the interfering chromogen appears in the urine, and this is in agreement with the opinion held by Goudsmit (1935) that the creatinine which is filtered at the glomerulus from the plasma will account for all that is found in the urine.

If the slope of this mathematical line, passing through the origin, represents the rate of glomerular filtration, then these lines strongly suggest that tubular excretion cannot play a significant rôle in the process of creatinine excretion. Claims to the contrary are based, largely on the work of Shannon (1935), whose results and conclusions will be discussed in the following section.

Discussion. The results obtained in the five experiments on those subjects in whom the renal clearance of creatinine was measured at various levels of plasma creatinine concentration, maintained approximately constant by continuous

infusion, are in general agreement with the results obtained after the ingestion of creatinine, after making a correction for the arterial and venous differences on a rising plasma curve. The creatinine clearance has been studied from endogenous levels of plasma (i.e. about 1mg./100 ml.) to 125 mg. per 100 ml, and the proportional relationship existing over this range shows that if there is any excretion of creatinine then the excretion moiety is extremely small.

The findings of Shannon, in so far as they might relate to the continuous infusion technique, are in complete disagreement with these results, since he found that the creatinine clearance fell as the plasma creatinine was raised to high levels. This led Shannon to believe that as a result of the reduction in the rate of excretion relative to the plasma level, a curvilinear relationship must then exist between UV and P, which is consistent with the theory of renal clearance, that tubular excretion must also be involved. If filtration alone were operative, this result would require that either the quantity of creatinine filtered decreased, or that the relative amount of creatinine reabsorbed (if any) increased as the plasma level rose. From a consideration of these two possibilities Shannon suggested that it appeared more likely that tubular excretion is involved; yet if tubular excretion of creatinine does occur, especially at

high plasma levels then the clearance should tend to rise, until a point is reached at which the clearance value is constant, indicating that the tubules are excreting to their full capacity. An analogous situation exists with regard to inulin, where, in two of the experiments the simultaneously determined inulin clearances had a tendency to rise relative to the highest plasma levels of inulin reached. This is in accordance with the work of Ferguson, Olbrich, Robson and Stewart (1949), who believe that inulin undergoes active reabsorption at low plasma levels and as the tubular cells attain the limits at which they are capable of absorbing inulin, then the observed inulin clearance tends to rise to a higher value, still below the creatinine clearance, yet with a definite tendency to approach this value. Further consideration of this point, however, reveals that in each of the two experiments, and at the point where the clearances showed this consistent rise, there was also a sharp rise in the rate of urine flow. This agrees with the accepted hypothesis that a more rapid flow along the tubules will reduce active reabsorption as well as back diffusion; it is also acknowledged that a water diuresis will also inhibit tubular reabsorption, e.g. sodium.

An examination of Shannon's results in the light of these experiments does reveal evidence of irregularities which can be explained and confirmed by experimental evidence

obtained from subjects who have been given creatinine by mouth. Shannon's experiments were not continuous infusion experiments in the sense that creatinine was injected intravenously, and maintained at an approximately constant plasma level of creatinine after allowing 30 minutes for equilibration. In his experiments Shannon initially gave 15 g. of creatinine by mouth, and then did three clearance periods; he then gave 10 g. creatinine intravenously, followed by a further three clearance periods, and finally, a second intravenous injection of 30 g. creatinine followed by three more clearance periods. In subject J.C. (see Table V) after the ingestion of the creatinine the clearances were 146.5, 153, 156; the clearances following the intravenous injections of creatinine were 144, 138.5, 134, and at the third level of creatinine 119, 136, 127. Preliminary experiments have shown that when creatinine is given by mouth, and depending on, to a large extent, when the venous blood is taken, since it appears to be a "hit or miss" procedure, the clearances are usually higher than the 'true' endogenous clearances, and on an average are 16 ml./min. higher (12 per cent) when the plasma level is rising. This discrepancy was found to be due to arterial and venous differences on a rising plasma curve, and could help to account for the high clearance values obtained by Shannon after ingestion of creatinine. A re-examination of his

figures and making a correction of 12 per cent for arterio-venous differences would bring the clearances down to 130, 137, 140, which is not significantly different from the clearance values obtained at the other two levels of creatinine except in one instance, 119. The important point is that with a rising plasma curve after the ingestion of creatinine the venous blood always contains less creatinine than the arterial blood and consequently all the clearance values tend to be too high.

It would appear that creatinine can diffuse quite freely through all cellular membranes, in contra-distinction to inulin. Obviously this must be so, otherwise creatinine formed in the muscle cells could not enter the blood stream to be excreted (diffusion coefficients for creatinine and inulin are 0.85 and 0.177. Smith and Smith, 1937). Evidence is also available to show that creatinine is equally distributed between the plasma and red blood cells, in relation to their respective water contents. But before creatinine becomes equally distributed between the plasma and extra-vascular fluids, following an intravenous injection, a certain period of time is required for this equilibrating process to take place. When the continuous infusion technique is employed, and maintained at a constant level of plasma creatinine, after allowing 30 minutes for equilibration, the clearance values are constant, and in good agreement with

the endogenous values. If, however, the plasma creatinine level is fluctuating, either up or down, then the clearance values are most irregular. This was very evident in one of the continuous infusion experiments (subject W.H.) where at the first exogenous level, all the clearance values tended to be high; the explanation being that sufficient time had not been allowed for an even distribution of the creatinine between the plasma and extra-cellular fluids. Homer Smith has also noticed that when the urine flow is suddenly increased, there is a suspicious increase in all the clearance values; he is, however, unable to offer any satisfactory explanation regarding this phenomenon.

On a falling plasma curve, however, the arterio-venous difference would not appear from the results in Table III to be significant. One might expect to find arterio-venous differences of the same order of magnitude on a falling curve as on a rising curve, but it is not necessarily true that creatinine passes into and out of cells with equal facility. Nevertheless there is a tendency for some of the clearances to fall slightly as the plasma creatinine falls and this cannot be explained on arterio-venous differences. The fall in the clearance values is, however, very small, and might be due to errors in ascertaining the mean plasma concentration at a time when the plasma creatinine level is falling quickly. The other two subjects studied by

Shannon, W.F. and S.C. show the same kind of behaviour and these results also can be explained by the same kind of reasoning.

The conclusions to be drawn from a reconsideration of these points would indicate that the results of Shannon could be interpreted as indicating a process of filtration with no tubular excretion. The so-called "self-depression" of the creatinine clearance as the plasma creatinine level rises would appear to be fictitious due largely to anamalies in the venous plasma creatinine concentration on a rising plasma curve after the ingestion of creatinine. Winkler and Parra (1937) also criticise the emphasis which Shannon attaches to the relation between the serum creatinine concentration and the creatinine/inulin ratio. They are of the opinion that this correlation is not justified by his results, since in the later stages of his experiments the serum creatinine level fell quite considerably without any change in the creatinine/inulin clearance ratio. A re-examination of Shannon's clearance figures, excluding the first oral level which is obviously incorrect, reveal very little difference, except one, in any of his clearance values.

The conclusion seems inescapably clear after considering the evidence of the continuous infusion experiments and the criticism of Winkler and Parra, that Shannon's results and

explanation are untenable, especially when he bases the the whole of his argument on the peculiar behaviour of the creatinine/inulin ratios at high plasma creatinine concentrations which were rapidly falling throughout the whole series of experiments, except the first level of creatinine, and which are also known to give erratic and unreliable results.

GENERAL DISCUSSION.

Rehberg based his hypothesis that creatinine is filtered by the glomeruli and is neither reabsorbed nor excreted by the tubules, upon the following points (Holten and Rehberg, 1931).

(I) Its concentration ratio is higher than that of any other urinary constituent.

(II) The excretion rate is independent of the volume of urine.

(III) Change in osmotic pressure of the plasma protein is associated with a change in the rate of creatinine excretion in the same direction as would be anticipated if the excreting process were one of ultrafiltration in the glomeruli.

Of these, the high concentration ratio and the constancy of excretion with changing urine volume strongly suggest that creatinine is not reabsorbed, but does not exclude the possibility that creatinine might be excreted in part by other mechanisms. The relation to plasma protein osmotic pressure, whilst affording evidence suggestive that some creatinine is filtered at the glomeruli, cannot be taken as evidence that creatinine is excreted solely in this manner.

In its broad outlines the filtration-reabsorption conception of renal function is firmly established on a sound

experimental and clinical basis. Also inherent in this theory is the assumption that the reabsorbed fluid remains constant in its composition, and also the denial of tubular excretion. Now, constancy in composition of the fluid removed from the filtrate by reabsorption implies that under changing conditions of urine flow the concentrations of the several low threshold substances should also remain constant, relatively to one another. Such a relationship does not hold, either in health or disease, but what is important is the fact, that whether the urine flow is slow or rapid creatinine excretion remains constant, whereas the quantity of urea excreted in the urine varies, more being returned to the blood in the reabsorbed fluid with slow than with rapid rates of urine flow, and the same might be true of other substances, e.g. inulin.

From Heidenhain's time to the present, innumerable experiments have been performed in attempts to prove or disprove whether the tubular cells are capable of excretion. And yet, the only available evidence for the tubular excretion of creatinine, in man, has been obtained by comparing the creatinine clearance with that of inulin, which is alleged to be a measure of glomerular filtration rate, yet has never been proved conclusively to be so.

If creatinine is wholly filtered by the glomeruli without alteration in concentration and is absolutely unaffected by

tubular activity then the amount excreted per minute (UV) would be a constant multiple of the plasma concentration (P) so that if UV were determined for various values of P, and UV were plotted graphically against P, the result would be a straight line passing through the origin. In all the continuous infusion experiments reported in this thesis, when UV was plotted against P the resulting line is straight and does go through the origin, and the endogenous clearance points on the graph (relatively very close to the origin) lie clearly on the line. In other experiments, when creatinine was given by mouth the true endogenous creatinine clearance was always identical with the exogenous creatinine clearance, using arterial blood at all attainable plasma levels of creatinine. The results also prove, that exogenous creatinine is filtered and excreted in precisely the same way, and at a rate which is dependent on the concentration of P, as endogenous creatinine. Again, since endogenous creatinine/inulin ratios are identical with exogenous creatinine/inulin ratios, using a continuous infusion of inulin, controversy over the discrepancy in the two ratios is resolved and shown to have been due largely, not so much to varying clearance values of inulin, but to incorrect determinations of 'true' creatinine. The identity of the two clearances further enhances the belief that creatinine is a measure of glomerular filtration rate in man.

This investigation has also shown most conclusively that an endogenous creatinine/inulin ratio of 1.0 is incorrect, due in many instances, to the non-creatinine chromogen being considered as creatinine.

From the foregoing statements it would appear that if a substance is to be a measure of glomerular filtration rate then under all conditions (e.g. rate of urine flow and plasma concentration) its clearance must always equal the rate of filtration. The chief factors in the regulation of reabsorption are: (a) the rate of flow along the tubules; the faster the flow, the shorter is the time permitted for reabsorption to occur, (b) when sufficient time is allowed, the osmotic pressure of the fluid in the tubules is the factor which brings reabsorption to an end. As a result of the reabsorption from the filtrate of a more dilute fluid than the filtrate, itself, concentration of non-threshold substances in the tubular fluid and a rise in its osmotic pressure must occur. Reabsorption ceases when the epithelial cells are incapable of performing the work necessary to overcome the osmotic resistance. Back diffusion of low threshold substances tends to increase, however, as the concentration rises. The concentration of the solute in the insterstitial fluids, i.e., the fluids on the other side of the tubular membrane, is a factor of equal importance. As this concentration approaches that of the tubular fluid, reabsorption becomes progressively lower and ultimately

ceases. It is, of course, impossible to exclude inulin from one of the above classes, and this might also be said of creatinine, but to a much less extent, if at all.

Even Shannon and Smith in their paper of 1935 state that the rate of glomerular filtration is at least as high as the level of the inulin clearance, but they admit that there is nothing to indicate the absence of active or passive reabsorption of this substance. They further point out that in view of the fact that evidence is at hand for the active reabsorption of xylose (and sucrose) further evidence (which is now available) is needed before the active reabsorption of inulin can definitely be ruled out. They say, too, that since the creatinine clearance can be depressed to within 10 per cent of the simultaneous inulin clearance, then the glomerular filtration rate cannot be higher than 10 per cent above the inulin clearance. This argument, however, is untrue, since this investigation has shown that there is no depression of the creatinine clearance, as the plasma creatinine is raised to very high levels.

Despite these positive findings other available evidence suggests that creatinine might be excreted by the tubules. For instance, the knowledge that phloridzin depresses the creatinine clearance in the dogfish to levels close to the sugar clearances (Clarke and Smith, 1932; Shannon, 1934) led Shannon to believe that if the creatinine in excess of

the inulin clearance, in man, is really excreted by the tubules then phloridzin in sufficiently high doses (100 mg./kilo) should produce this desired effect. He found that the clearances of creatinine and inulin did come together presumably by a specific depressant action on the tubular excretion of creatinine. Shannon's figures, however, also show a significant fall in all the clearances after phloridzin so this argument is open to serious criticism. As Homer Smith (1951) has pointed out there also remains the possibility that phloridzin may injure the tubules and that substances, e.g. glucose, escape by diffusion, also that phloridzin may impair tubular reabsorption or excretion of other substances, or indeed alter the filtration rate by action on the glomerular arterioles. Poisoning of the tubule, however, though it abolishes the specific reabsorptive process permits water and other substances to pass back into the blood by a simple process of diffusion, and its use is therefore very limited.

Crawford (1948) has shown that the creatinine/inulin ratio is depressed to or towards 1.0 by the administration of large doses of diodone or para-amino hippuric acid, and considers it additional evidence for the tubular excretion of creatinine in man. It should be pointed out, again, that her results are also open to the same criticism as Shannon's phloridzin results, namely, that the results show

a significant fall in all the clearances after diodone and para-amino hippuric acid, and therefore might be interpreted as indicating tubular injury and back diffusion. Tubular excretion of substances (other than H^+ ions), e.g. ammonia, and hippuric acid which are formed by the kidney and passed into the urine, if a normal kidney function, appears to involve only two transport systems with a factor common to both; hence loading the tubules with diodone or para-amino hippuric acid to measure Tm_D and Tm_{PAH} is known to depress the excretion of all substances in one or other of two groups. The suggestion has been made that tubular reabsorption and tubular excretion may be mutually competitive for it has been found repeatedly that the simultaneous measurement of Tm_{PAH} and Tm_G gives low values for both, but this is just speculation.

In a consideration of this evidence, for and against the reliability of creatinine as a measure of glomerular filtration rate, must also be included inulin, for in the minds of many workers there are still very grave doubts as to its reliability. Therefore, for people to compare creatinine clearances, also clearance values obtained by the use of other substances, with inulin clearances, and so decide whether there is excretion or reabsorption on the grounds that inulin does measure glomerular filtration

rate is extremely dangerous, and in the light of what is now known regarding inulin, a certain amount of caution has to be exercised in the interpretation of such clearances. The facts as found in this investigation do strongly suggest that creatinine, if not a true measure of glomerular filtration rate is very much nearer to this value than inulin. Two of the experiments have given data of the kind which led other workers (Ferguson, Olbrich, Robson and Stewart, 1949) to the belief that inulin undergoes active reabsorption or back diffusion by the tubules. If creatinine does undergo passive reabsorption, then it must be almost negligible, since the creatinine clearance in normal subjects is roughly 30-40 per cent higher than the simultaneously determined inulin clearance, which too is alleged to undergo reabsorption to the extent of 15 per cent of more (Ferguson, Olbrich, Robson and Stewart, 1949).

The question of tubular excretion may be summed up by saying that, though foreign substances, e.g. dyes, diodone, penicillin, etc., are excreted by the proximal tubules of the mammalian kidney, such a process for substances which are normally present in the glomerular filtrate, is probably of relatively little importance under ordinary physiological circumstances. It seems unnecessary to invoke tubular excretion to explain the several phases of renal activity, nor does such a conception aid greatly in the interpretation

of renal disease. A consideration of renal clearance tests from this point of view, also indicates that creatinine clearances are preferable to inulin clearances. Glomerular damage and reduction in filtration rate go hand in hand with the ability of the kidney to clear the blood of e.g., creatinine, urea. Also, in acute nephritis the glomeruli are chiefly involved in the inflammatory process, filtration is reduced and retention of creatinine and urea in the blood may be a pronounced feature; in lipoid nephrosis, on the other hand, there is little or no detectable injury to the glomeruli, whereas the tubular cells show striking degenerative changes. Filtration is apparently unimpaired and creatinine and urea retention does not occur, which one should expect were the excretion of these substances an important duty of the tubules. On the other hand, it is quite possible if not probable, that in chronic kidney disease involving the glomeruli (glomerulo-nephritis) the primitive excretory functions of the tubules may be called upon to play a much more important rôle than they do in health.

SUMMARY

PART I

1. The results using the enzymatic method of Miller and Dubos show that approximately 80 per cent of the total chromogenic material in normal serum is true creatinine and 57 per cent of that in cells is true creatinine. Urine contains approximately 96 per cent true creatinine.
2. Erythrocytes contain on an average 43 per cent of non-creatinine material, but the true creatinine is of the same order as in serum.
3. Kaolin adsorbs creatinine quantitatively from serum filtrates and from pure solutions, and the results show that approximately 75-80 per cent of the total chromogenic material in normal serum is true creatinine.
4. The interfering chromogen may be aceto-acetic acid.

PART II

1. The 'true' endogenous creatinine clearance is invariably higher than the simultaneous inulin clearance by 30-40 per cent.
2. The 'true' endogenous creatinine/inulin ratio is always greater than 1.0.
3. The exogenous creatinine/inulin ratio is also greater than 1.0.
4. The 'true' endogenous creatinine clearance equals the exogenous creatinine clearance.

5. When creatinine is given by mouth the exogenous creatinine clearance is always higher than the 'true' endogenous creatinine clearance. The discrepancy in the two values has been found to be due to differences in arterial and venous blood concentration.

6. The continuous infusion experiments have shown that there is a direct proportionality between UV and P between endogenous plasma levels of creatinine and 125 mg./100 ml. serum.

7. The results of all the experiments are held to suggest strongly that, in man, both 'endogenous' and 'exogenous' creatinine are filtered by the glomeruli, and neither undergo reabsorption nor excretion by the tubular cells.

8. The results also strongly suggest that both endogenous and exogenous creatinine are, in man, a true measure of glomerular filtration rate, and that inulin is not a true measure of glomerular filtration rate, since there is very good evidence to believe that inulin undergoes reabsorption by the tubular cells.

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